

1-1-2018

Addressing The Threat Of Multidrug Resistant Infectious Diseases By Synthesis Of Novel Aminoglycoside Antibiotics

Girish Chandra Sati
Wayne State University,

Follow this and additional works at: https://digitalcommons.wayne.edu/oa_dissertations



Part of the [Organic Chemistry Commons](#)

Recommended Citation

Sati, Girish Chandra, "Addressing The Threat Of Multidrug Resistant Infectious Diseases By Synthesis Of Novel Aminoglycoside Antibiotics" (2018). *Wayne State University Dissertations*. 1958.
https://digitalcommons.wayne.edu/oa_dissertations/1958

This Open Access Embargo is brought to you for free and open access by DigitalCommons@WayneState. It has been accepted for inclusion in Wayne State University Dissertations by an authorized administrator of DigitalCommons@WayneState.

**ADDRESSING THE THREAT OF MULTIDRUG RESISTANT INFECTIOUS
DISEASES BY SYNTHESIS OF NOVEL AMINOGLYCOSIDE ANTIBIOTICS**

by

GIRISH CHANDRA SATI

DISSERTATION

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

2018

MAJOR: CHEMISTRY (Organic)

Approved By:

Advisor	Date
---------	------

DEDICATION

I dedicate my PhD work to my parents Mr. Lalita Prasad and Mrs. Leela Devi, my siblings and friends for their endless love, support and guidance.

ACKNOWLEDGEMENTS

First of all, I would like to express my deep admiration to my advisor, Professor David Crich, for his constant motivation, guidance, support and patience during the course of my researches in his laboratory. His passion and diligence for new scientific discoveries always encourage me to do better. Without his timely input and support, it would have been extremely difficult to finish this thesis.

I am extremely thankful to our collaborators, Professors Andrea Vasella and Erik C. Böttger, for their enormous contribution to my research work. It would have been very difficult to make progress in this project without their expertise in the field and timely suggestions. I would also like to thank all my committee members, Professors James H. Rigby and Vladimir Chernyak and Dr. Neil Price, for their precious time and suggestions during my doctoral studies. My sincere thanks to Professors Cha, Guo, Kodanko, Stockdill, Ahn, and Winter, who taught me various courses during my first-year. The knowledge acquired in those courses was the foundation for my research conducted in last five years.

I would like to extend my appreciation to my present and past lab mates, Dr. P. Kancharla, Dr. T. Kato, Dr. T. Furukawa, Dr. S. Mondal, Dr. T. Matsushita, Dr. R. Salla, Dr. S. Dharuman, Dr. S. Buda, Dr. O. Popik, Dr. A. R. Mandhapati, Dr. C. Navuluri, Dr. M. Moumé-Pymbock, Dr. V. Sarpe, Dr. P. Rajasekaran, Dr. A. Sonousi, Peng, Harsha, Philip, Jessica, Bibek, Sandeep, Xiaoxiao, Guanyu, Mike, Mohamed, Philemon, Nuwan, Jonny, Sameera, Timothy, Jonathan, Benjamin, Brendan, Daren, Guillaume, James, Dean, and Shuay for their timely help and important suggestions in the lab. Finally, I would like to thank the helpful staff in the chemistry department, as well as my supportive friends and family.

TABLE OF CONTENTS

DEDICATION	ii
ACKNOWLEDGEMENTS	iii
LIST OF FIGURES	vii
LIST OF SCHEMES.....	ix
LIST OF TABLES	xi
LIST OF ABBREVIATIONS.....	xiii
CHAPTER 1. AMINOGLYCOSIDE ANTIBIOTICS (AGAs).	1
1.1. General Introduction:	1
1.2. Chemical Structure of AGAs:	6
1.3. Mode of action of AGAs:	8
1.4. Toxicity and selectivity of AGAs:	13
1.4.1. Nephrotoxicity:.....	13
1.4.2. Ototoxicity:.....	14
1.4.3. Selectivity of AGAs:	16
1.5. Bacterial resistance to AGAs:	19
1.5.1. Decreased uptake of AGAs:	19
1.5.2. AGA efflux:.....	20
1.5.3. Target modification:	21
1.5.4. Aminoglycoside modifying enzymes (AMEs):.....	22
1.5.5. Strategies to overcome aminoglycoside resistance:	25
1.6. Goals:	28
CHAPTER 2. MODIFICATIONS TO NEOMYCIN B.	29
2.1. N6'-, N6'''-, and O4'-modifications to neomycin B.	29

2.1.1. Introduction:	29
2.1.2. Synthesis:.....	30
2.1.3. Biological evaluations:	35
2.1.4. Conclusion:.....	43
2.2. <i>N2'</i> -modifications to neomycin B.....	44
2.2.1. Introduction:	44
2.2.2. Synthesis:.....	45
2.2.3. Biological evaluations:	54
2.2.4. Conclusion:.....	60
CHAPTER 3. MODIFICATIONS TO PAROMOMYCIN.	61
3.1. <i>N2'</i> -modifications to paromomycin.	61
3.1.1. Introduction:	61
3.1.2. Synthesis:.....	62
3.1.3. Biological evaluations:	65
3.1.4. Conclusion:.....	68
3.2. 5''-modifications to paromomycin.	69
3.2.1. Introduction:	69
3.2.2. Synthesis:.....	72
3.2.3. Biological evaluations:	76
3.2.4. Conclusion:.....	79
CHAPTER 4. SYNTHESIS OF 3- <i>N</i> -ALKYL PYRIMIDIN-2,4-DIONE DERIVATIVES.	81
5.1. Introduction:.....	81
5.2. Synthesis:	82
5.3. Discussion:	87
5.4. Conclusion:	90

CHAPTER 5. OVERALL CONCLUSIONS.....	91
CHAPTER 6. EXPERIMENTAL SECTION.....	94
REFERENCES	151
ABSTRACT.....	168
AUTOBIOGRAPHICAL STATEMENT.....	171

LIST OF FIGURES

Figure 1. Cell wall structure of Gram-positive and Gram-negative bacteria.	2
Figure 2. Dimeric and pentameric structure of salvarsan (arsphenamine).	2
Figure 3. Penicillin-G and Streptomycin: starting points of the antibiotic era.	4
Figure 4. Timelines for the discovery of new classes of antibiotics.	5
Figure 5. Plazomicin, a new generation of AGA derived from sisomicin.	6
Figure 6. Neomycin and Kanamycin classes of aminoglycosides.	7
Figure 7. Some AGAs with unusual structures.	8
Figure 8. Purine and pyrimidines bases present in nucleic acids and Watson-Crick base pairs....	9
Figure 9. Schematic representation of protein synthesis.	10
Figure 10. Schematic representation of AGA interfering with the protein synthesis.	11
Figure 11. Pseudo base-pair type interaction between ring-I of paromomycin	12
Figure 12. Schematic representation of paromomycin (4,5-series AGA) interactions with bacterial 16S r-RNA.	12
Figure 13. Schematic representation “flipped-in” and “flipped-out” conformation of adenines in bacterial A-site.	13
Figure 14. Origin dependent differences in the r-RNA decoding A-Site (AGA binding site is shown in the box).	17
Figure 15. Origin dependent differences in the r-RNA decoding A-Site (Bacterial numbering is used throughout for clarity and A1555 in the mitochondrial numbering corresponds to A1490 in the bacterial numbering).	18
Figure 16. Potential modification sites of streptomycin, kanamycin B, neomycin B,	23
Figure 17. Structures of gentamicin, tobramycin, and amikacin.	25
Figure 18. Some of the AME inhibitors.	26
Figure 19. Some of the rationally designed semisynthetic AGAs.	27
Figure 20. Structures of neomycin B, paromomycin, 4'-O-ethyl paromomycin, sisomicin, and plazomicin.	30

Figure 21. Schematic representation of intramolecular 2'-5''-hydrogen bond in neomycin B and its interactions with bacterial 16S r-RNA.	45
Figure 22. Structures of 2'- <i>N</i> -formyl neomycin B 76 and 2'- <i>N</i> -acetyl neomycin B 77	53
Figure 23. Some of the 5''-modified derivatives of paromomycin, lividomycin B, neomycin B, and butirosin A.	71
Figure 24. Neomycin B dimer and neomycin B-anthraquinone conjugated linked at 5''-position.	72
Figure 25. Cis (E)- and trans (Z)-configurations in <i>N</i> -monosubstituted amides.	76

LIST OF SCHEMES

Scheme 1. Synthesis of 4'- <i>O</i> -ethyl neomycin B 27	32
Scheme 2. Synthesis of neomycin B derivatives 37 , 38 and 42	33
Scheme 3. Synthesis of neomycin B derivatives 44 and 46	34
Scheme 4. Synthesis of neomycin B derivatives 53 , 54 , and 56	38
Scheme 5. Synthesis of 2'- <i>N</i> -alkyl neomycin B derivatives 59 and 61	48
Scheme 6. Synthesis of 2'-deamino neomycin B 64	49
Scheme 7. Proposed radical mechanism for replacement of isonitrile with a hydrogen atom (R-NC represents a fully acetylated neomycin B derivative with a isonitrile group at 2'-position).. 50	
Scheme 8. Synthesis of glycosyl acceptor 67	51
Scheme 9. Proposed mechanism for the formation of glycosyl acceptor 67 from the 2'-amino intermediate (R = ring II, III and IV of paromomycin derivative 66).	52
Scheme 10. Synthesis of 2'-deamino-2'-hydroxy neomycin B 70	52
Scheme 11. Proposed mechanism for DMF mediated α -selective glycosylation.	53
Scheme 12. Synthesis of 2'- <i>N</i> -(2-aminoacetyl)-neomycin B 75	54
Scheme 13. Synthesis of 2'- <i>N</i> -alkyl paromomycin derivatives 79 , 81 and 83	63
Scheme 14. Synthesis of 2'-deamino-2'-hydroxy paromomycin 86	64
Scheme 15. Synthesis of 5''-deoxy-5''-amino intermediate 108	73
Scheme 16. Synthesis of 5''-modified paromomycin derivatives 110 , 112 , and 114	74
Scheme 17. Unexpected formation of the 3- <i>N</i> -alkyl pyrimidin-2,4-dione derivative 118	82
Scheme 18. Overall synthesis of the 3- <i>N</i> -alkyl pyrimidin-2,4-dione derivatives from maleic anhydrides.	82
Scheme 19. Synthesis of <i>N</i> -hydroxy maleimide derivatives 122 and 123	83
Scheme 20. Synthesis of <i>N</i> -hydroxymaleimide derivative 127	83
Scheme 21. Synthesis of <i>N</i> -hydroxymaleimide derivative 130	84

Scheme 22. Synthesis of <i>N</i> -trifloxymaleimide derivatives 131 , 115 , 132 , and 133	84
Scheme 23. Common reaction procedure for the synthesis of 3- <i>N</i> -alkyl pyrimidin-2,4-dione derivatives.	85
Scheme 24. Reaction mechanism for the formation of the 3- <i>N</i> -alkyl pyrimidin-2,4-dione.....	88
Scheme 25. Formation of 3- <i>N</i> -benzoyloxy quinazolinedione 151	89
Scheme 26. Formation of 3- <i>N</i> -benzenesulfonyloxy dihydrouracil 154	89
Scheme 27. Lossen rearrangement of <i>N</i> -toluenesulfonyloxy phthalimide 157 and maleimide derivative 160	90

LIST OF TABLES

Table 1. Antiribosomal activities (IC ₅₀ , µg/mL) and selectivities of modified neomycin B derivatives (selectivities are obtained by dividing the eukaryotic values by the bacterial values).	39
Table 2. Change in antiribosomal selectivity (selectivity quotient = selectivity of derivative/selectivity of parent) of neomycin B derivatives with respect to parent neomycin B.	40
Table 3. Antibacterial activities of modified neomycin B derivatives (MIC, µg/mL).	41
Table 4. Antibacterial activities (MIC, µg/mL) of modified neomycin B derivatives against ESKAPE pathogens.	41
Table 5. Antibacterial data of modified neomycin B derivatives against wild type and engineered strains of <i>E. coli</i> carrying specific resistance determinants (MIC, µg/ml).	43
Table 6. Antiribosomal activities (IC ₅₀ , µM) and selectivities of 2'-modified neomycin B derivatives (selectivities are obtained by dividing the eukaryotic values by the bacterial values).	56
Table 7. Change in antiribosomal selectivity (selectivity quotient = selectivity of derivative/selectivity of parent) of 2'-modified derivatives with respect to parent neomycin B..	58
Table 8. Antibacterial activities of 2'-modified neomycin B derivatives (MIC, µg/mL).....	58
Table 9. Antibacterial data of 2'-modified neomycin B derivatives against wild type and engineered strains of <i>E. coli</i> carrying specific resistance determinants (MIC, µg/ml).....	59
Table 10. Antiribosomal activities (IC ₅₀ , µg/mL) and selectivities of 2'-modified paromomycin derivatives (selectivities are obtained by dividing the eukaryotic values by the bacterial values).	66
Table 11. Change in antiribosomal selectivity (selectivity quotient = selectivity of derivative/selectivity of parent) of 2'-modified derivatives with respect to parent paromomycin.	66
Table 12. Antibacterial activities of 2'-modified paromomycin derivatives (MIC, µg/mL).	67
Table 13. Antibacterial data of 2'-modified paromomycin derivatives against wild type and engineered strains of <i>E. coli</i> carrying specific resistance determinants (MIC, µg/ml).....	68
Table 14. Percentage of cis- and trans-conformers in various <i>N</i> -monosubstituted amides.	75
Table 15. Antiribosomal activities (IC ₅₀ , µg/mL) and selectivities of 5''-modified paromomycin derivatives (selectivities are obtained by dividing the eukaryotic values by the bacterial values).	77

Table 16. Change in antiribosomal selectivity (selectivity quotient = selectivity of derivative/selectivity of parent) of 5''-modified derivatives with respect to parent paromomycin.	78
Table 17. Antibacterial activities of 5''-modified paromomycin derivatives (MIC, µg/mL).....	79
Table 18. Antibacterial data of 5''-modified paromomycin derivatives against wild type and engineered strains of <i>E. coli</i> carrying specific resistance determinants (MIC, µg/ml).....	79
Table 19. Examples of synthesized 3- <i>N</i> -alkyl pyrimidin-2,4-dione derivatives.....	85

LIST OF ABBREVIATIONS

A	Adenine
AAC	Aminoglycoside acetyltransferases
Ac	Acetyl
ACN	Acetonitrile
AcOH	Acetic acid
ADP	Adenosine diphosphate
AGA	Aminoglycoside antibiotics
AIBN	Azobisisobutyronitrile
AME	Aminoglycoside modifying enzyme
ANT	Aminoglycoside nucleotidyltransferases
APH	Aminoglycoside acetyltransferases
Ar	Aryl
ATP	Adenosine triphosphate
Boc	tert-Butyloxycarbonyl
Bn	Benzyl
Bu	Butyl

Bz	Benzoyl
c	Concentration
C	Cytosine
°C	Celsius
Calcd.	Calculated
Cbz	Benzyloxycarbonyl
COSY	Homonuclear correlation spectroscopy
m-CPBA	<i>m</i> -Chloroperbenzoic acid
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOS	Deoxystreptamine
EDP	Energy-dependent phase

EDC	<i>N</i> -(3-Dimethylaminopropyl)- <i>N'</i> -ethylcarbodiimide
ESI	Electrospray ionization
ESIHRMS	Electrospray ionization high resolution mass spectrometry
Et	Ethyl
Et ₃ N	Triethylamine
EtOH	Ethanol
EtOAc	Ethyl acetate
FT/IR	Fourier transform infrared
G	Guanine
h	Hour
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple bond correlation
HMPA	Hexamethylphosphoramide
HOBt	1-Hydroxybenzotriazole
HSQC	Heteronuclear single quantum coherence
Hz	Hertz
LPS	lipopolysaccharides

MDR	Multi-drug-resistant
Me	Methyl
MeOH	Methanol
mmol	Millimole
mp	Melting point
mRNA	Messenger ribonucleic acid
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MS	Molecular sieves
Ms	Methanesulfonyl
NADPH	Nicotinamide adenine dinucleotide phosphate
NBS	<i>N</i> -Bromosuccinamide
NIS	<i>N</i> -Iodosuccinamide
NMR	Nuclear magnetic resonance
nOe	Nuclear Overhauser effect
NOS	<i>N</i> - <i>O</i> -succinimide
OHC	Outer hair cells
Ph	Phenyl

PMe ₃	Trimethylphosphine
ppm	Parts per million
pTSA	4-Toluene sulfonic acid
Py	Pyridine
ROS	Reactive oxygen species
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RT	Room temperature
Stick's reagent	Imidazole-1-sulfonyl azide hydrochloride
TB	Tuberculosis
TEA	Triethylamine
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Trisyl	2,4,6-Trisopropylbenzenesulfonyl

tRNA	Transfer ribonucleic acid
Ts	p-Toluenesulfonyl
TTMS	Tris(trimethylsilyl)silane
U	Uracil
WHO	World health organization

CHAPTER 1. AMINOGLYCOSIDE ANTIBIOTICS (AGAs).

1.1. General Introduction:

Bacteria constitute a large domain of prokaryotic microorganisms and were one of the first life forms to appear on earth. The Dutch microbiologist Antonie Van Leeuwenhoek first observed bacteria in 1776.¹ In 1884 Danish bacteriologist Hans Christian Gram developed a method for staining bacteria, to make them more visible under a microscope and this became very useful in classifying bacteria. A Gram stain is made using a primary stain of crystal-violet and a counterstain of safranin. Bacteria that turn purple when stained are called “Gram-positive” while those that turn red when counterstained are called “Gram-negative”.² The basic difference between Gram-positive and Gram-negative bacteria is the structure of their cell wall. In Gram-positive bacteria, the cell wall consists of a thick peptidoglycan layer and a plasma membrane. On the other hand, in Gram-negative bacteria, the cell wall composed of a thinner peptidoglycan layer sandwiched between an inner plasm membrane and an outer membrane (Figure 1).³ When the bacteria form a parasitic association with other organisms, they are classified as pathogens. Pathogenic bacteria are the major cause of human diseases and many lethal diseases like tetanus, typhoid, diphtheria, syphilis, cholera, leprosy, tuberculosis, pneumonia etc., are caused by bacterial infection. Diseases resulting from pathogenic bacteria are one of the leading causes of human mortality and tuberculosis is top of the list. According to “Global Tuberculosis Report 2016” published by WHO,⁴ there were an estimated 10.4 million new cases of TB and 1.8 million deaths worldwide in the year 2015 alone. TB is the leading killer of HIV-positive people and in 2015, 35% of HIV deaths were due to TB. Globally in 2015, an estimated 580,000 people developed multidrug-resistant tuberculosis (MDR-TB) and TB remained one of the top 10 causes of human death.

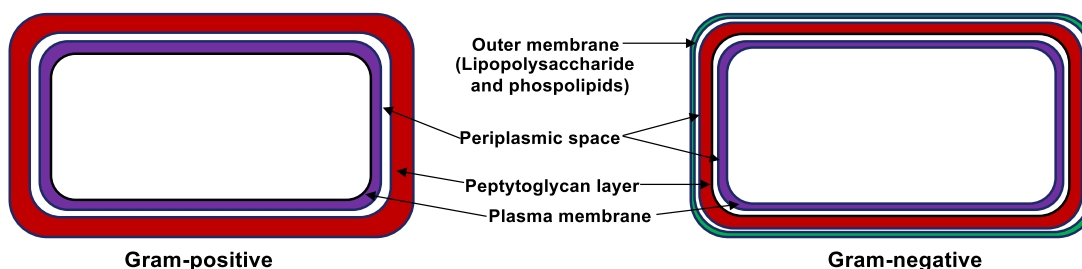


Figure 1. Cell wall structure of Gram-positive and Gram-negative bacteria.

Antibiotics are the weapons discovered by humans to fight against bacteria. An antibiotic is a substance which either kills or inhibits the growth of bacteria. Bactericidal antibiotics kill the bacteria whereas bacteriostatic antibiotics inhibit the rapid growth of the bacteria leaving it to the host's immune system to clear the bacteria. It wasn't until the late 19th century that scientists started observing the antimicrobial action of chemicals. Initially the German physician Paul Ehrlich noted that certain chemical dyes colored some bacterial cells but not others; a precursor technique to gram staining and proposed that it might be possible to create substances that can kill certain bacteria without affecting other cells. In 1907 his laboratory synthesized a chemical salvarsan now called arsphenamine (Figure 2) that was an effective treatment for syphilis.⁵⁻⁷ This organoarsenic compound was the first modern chemotherapeutic agent. The Ukrainian-American biochemist and microbiologist Selman Waksman first used the term antibiotic in 1942.⁸

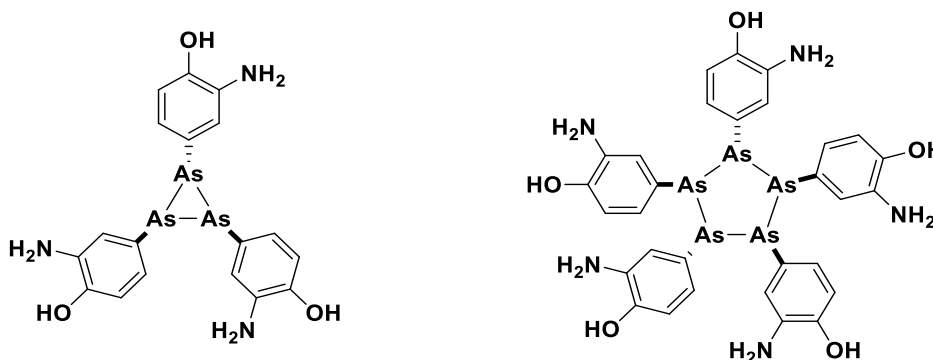


Figure 2. Dimeric and pentameric structure of salvarsan (arsphenamine).⁹

Penicillin-G was the first antibiotic discovered by Scottish biologist Sir Alexander Fleming in 1928.¹⁰ It was isolated from *Penicillium* fungi and used for the treatment of a number of bacterial infections. The discovery of Penicillin-G started the antibiotic era and opened the door for the development of several structurally similar derivatives to treat many other bacterial infections. Since the discovery of penicillin, various other types of antibiotics emerged including other β -lactams, aminoglycosides, tetracyclines, quinolones etc. Antibiotics can be divided into four categories based on their mode of action:³

1. Cell wall biosynthesis inhibitors (e.g. penicillins, cephalosporins).
2. Protein biosynthesis inhibitors (e.g. aminoglycosides, tetracyclines, oxazolidinones, macrolides).
3. DNA replication and repair inhibitors (e.g. quinolones, fluoroquinolones).
4. Folate coenzyme biosynthesis inhibitors (e.g. sulfonamides).

AGAs belong to the category of antibiotics that inhibit bacterial protein synthesis. AGAs are highly potent broad spectrum antibiotics and have much desired bactericidal activity. Streptomycin, the first AGA, was discovered by Selman Waksman in 1944. Its discovery was a landmark in antibiotic history and it was used for decades for the treatment of TB caused by *Mycobacterium tuberculosis*.¹¹ Streptomycin is a bactericidal antibiotic and was isolated from the Actinobacterium *Streptomyces griseus*. Most of the AGAs are produced by *Actinomycetes* of either genus *Streptomyces* (AGAs named as “mycin”) or *Micromonospora* (AGAs named as “micin”). AGAs are particularly effective against Gram-negative bacteria.

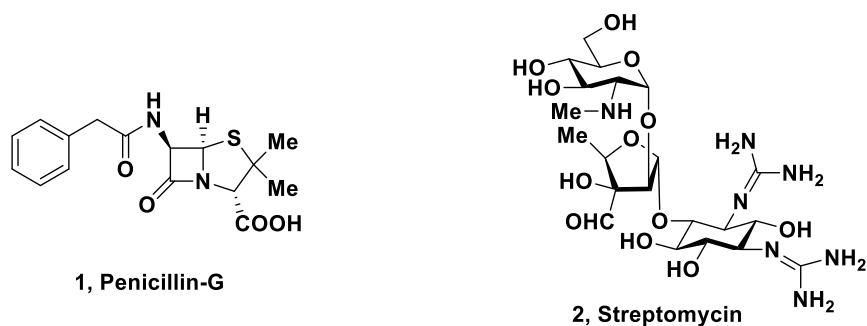


Figure 3. Penicillin-G and Streptomycin: starting points of the antibiotic era.

AGAs are listed by the WHO as one of the critically important antimicrobials and have long been used as highly potent broad-spectrum antibiotics against Gram-negative pathogens, methicillin-resistant *Staphylococcus aureus* (MRSA), and multidrug-resistant tuberculosis (MDR-TB) as well as to treat many other complex infectious diseases. The AGAs form a large family of water soluble, polycationic amino sugars that inhibit the protein synthesis by direct interaction with ribosomal RNA which leads to the bacterial cell death. AGAs are given intravenously because of their poor absorbance from the gastrointestinal tract. Due to their significant toxic effects, in particular irreversible hearing loss (ototoxicity) and kidney damage (nephrotoxicity), and with the development of new orally administered antibiotics, interest in development of AGAs declined in 1970s. In recent years, there is much interest in the development of new generation of AGAs for multiple reasons.¹²⁻¹⁴ First, the increase in antibiotic resistant diseases has limited the use of available antibiotics and AGAs remain very crucial for the treatment of those diseases.¹⁵⁻¹⁷ Second, the mechanism of AGA antibacterial action and the mechanisms of resistance are well-studied, which provides a strong basis for rational design of a new generation of resistance proof and less toxic AGAs.¹⁸⁻³¹ Third, there are significant challenges in discovering a new class of antibiotics and bacterial metabolism offers only a limited number of targets suitable for antibiotic development. Because of these significant hurdles in the antibiotics discovery, only two new

classes of antibiotics have been developed since the 1970s (Figure 4).³² Finally, the low cost of naturally occurring AGAs and the well-developed AGA chemistry enables the easy synthesis of novel AGAs.^{25,33,34} Numerous strategies have been applied in recent years to circumvent AGA resistance³⁵ and the ongoing renewal of AGA research has yielded plazomicin, a semisynthetic derivative of the natural AGA sisomicin, which is currently in phase III clinical trials for complicated urinary tract infections (Figure 5).^{36,37}

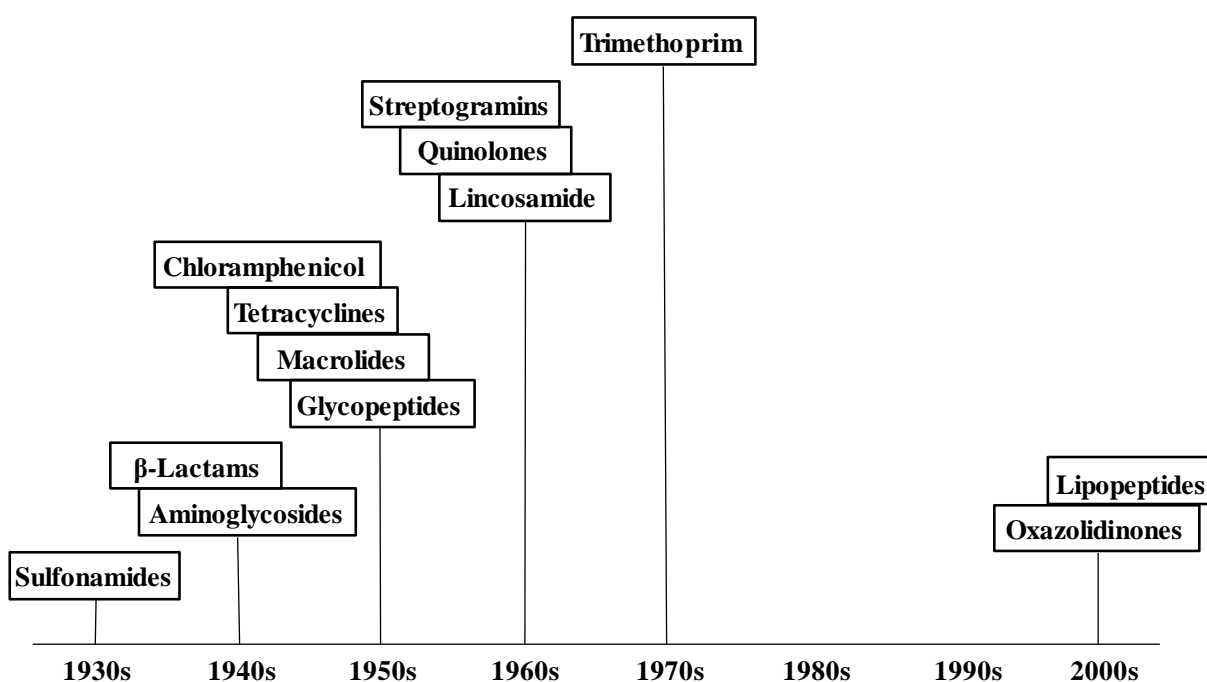


Figure 4. Timelines for the discovery of new classes of antibiotics.

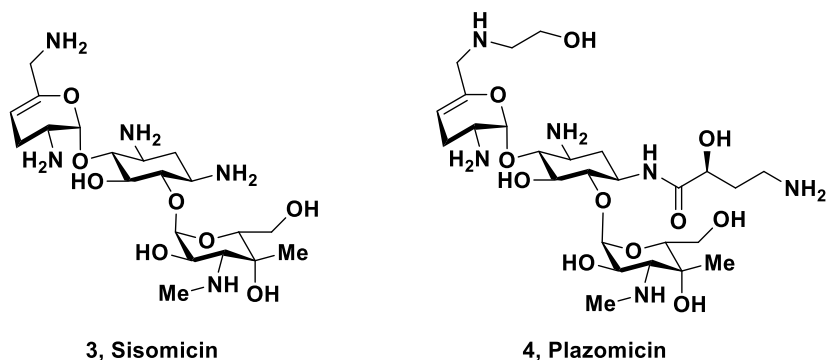
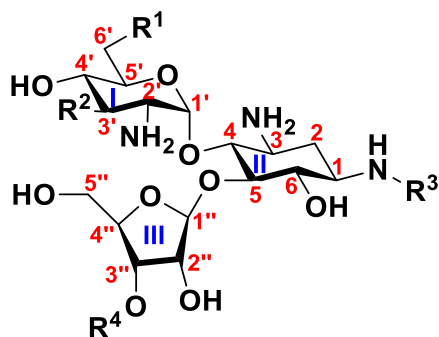


Figure 5. Plazomicin, a new generation of AGA derived from sisomicin.

1.2. Chemical Structure of AGAs:

Structurally AGAs are amino modified sugars linked through glycosidic bonds. Most of the AGAs have a backbone structure consisting of an aminocyclitol linked to other amino sugars through glycosidic bonds. In most of the clinically useful AGAs, the aminocyclitol is disubstituted 2-deoxystreptamine (DOS). Streptomycin has a monosubstituted streptamine core where amino groups are modified to guanidino groups. Beside streptomycin, neomycin and kanamycin are the two most studied AGAs. Neomycin belongs to a group of AGAs containing a 4,5-disubstituted 2-deoxystreptamine core (ring-II), while kanamycin contains a 4,6-disubstituted 2-deoxystreptamine core (Figure 6).²⁵ However, other AGAs have been discovered whose unusual structures do not fit the classification (Figure 7). Aparamycin is one such unusual monosubstituted 2-deoxystreptamine AGA that is produced by *Streptomyces tenebrarius*.³⁸ It causes little or no ototoxicity in animal models^{39,40} and avoid modifications by most aminoglycoside modifying enzymes, thereby retaining activity against multidrug resistant pathogens. Hygromycin B, produced by a bacterium *Streptomyces hygroscopicus* and isolated in 1953 from a soil sample, has an unusual 5-substituted 2-deoxystreptamine structure.⁴¹ It is added to swine and chicken feed as an anthelmintic or anti-worming agent. Another example is spectinomycin, which consists of three fused rings and whose

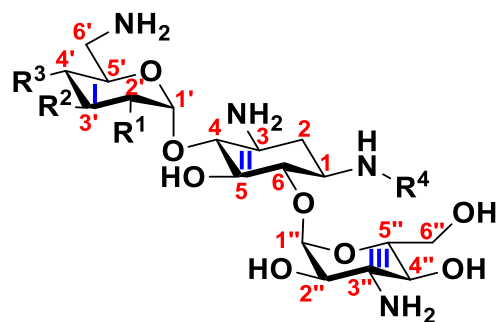
aminocyclitol ring is called spectinamine.^{27,42} Spectinomycin was discovered in 1961 and is industrially produced by the fermentation of the bacterium *Streptomyces spectabilis*. Spectinomycin is a very useful antibiotic for the treatment of gonorrhea, especially in patients who are allergic to penicillin or cephalosporin.⁴³



	R ¹	R ²	R ³	R ⁴
Neomycin B	NH ₂	OH	H	DAG
Paromomycin I	OH	OH	H	DAG
Lividomycin B	OH	H	H	DAG
Butirosin B	NH ₂	OH	AHB	H
Ribostamycin	NH ₂	OH	H	H

DAG: 2,6-diamino-2,6-dideoxy- α -L-idoopyranosyl

4,5-AGA Series (Neomycin Class)



	R ¹	R ²	R ³	R ⁴
Kanamycin A	OH	OH	OH	H
Kanamycin B	NH ₂	OH	OH	H
Dibekacin	NH ₂	H	H	H
Tobramycin	NH ₂	H	OH	H
Amikacin	OH	OH	OH	AHB
Arbekacin	NH ₂	H	H	AHB

AHB:(S)-4-amino-2-hydroxybutyryl

4,6-AGA Series (Kanamycin Class)

Figure 6. Neomycin and Kanamycin classes of aminoglycosides.

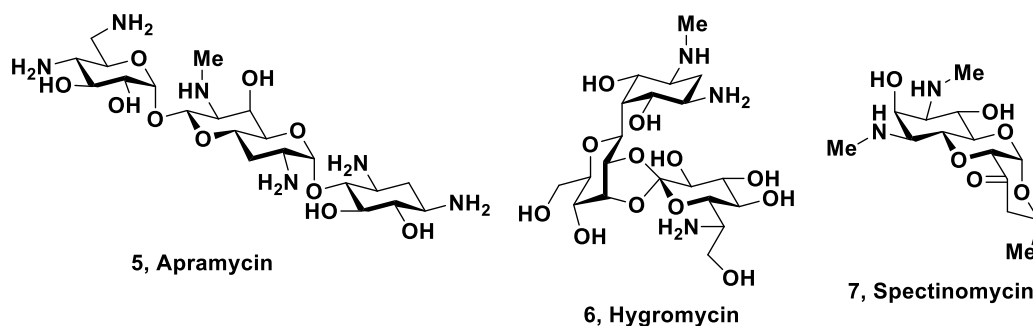


Figure 7. Some AGAs with unusual structures.

1.3. Mode of action of AGAs:

Nucleotides have various roles in cellular mechanisms and are the constituents of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleotides have three basic components: (i) a nitrogenous base (pyrimidine and purine base), (ii) a pentose sugar (deoxyribose in DNA and ribose in RNA), and (iii) a phosphate. Nucleic acids are the polymeric structures of the nucleotides joined in specific sequences through a covalent phosphodiester linkage and called polynucleotides. DNA and RNA both have two purine bases, adenine (A) and guanine (G). In both DNA and RNA one of the pyrimidine bases is cytosine (C); while the other is thymine (T) in DNA and uracil (U) in RNA. The ring exo and endocyclic amines and carbonyl groups present on the nitrogenous bases are such that specific pairs of bases form very crucial hydrogen-bonding interactions with each other. These important hydrogen bonding interactions were discovered by Watson and Crick in 1953, thus A pairs specifically with T or U and G pairs specifically with C. These two types of base pairing are responsible for the double stranded structure of DNA and parts of RNA and underlie the duplication of genetic information (Figure 8).⁴⁴

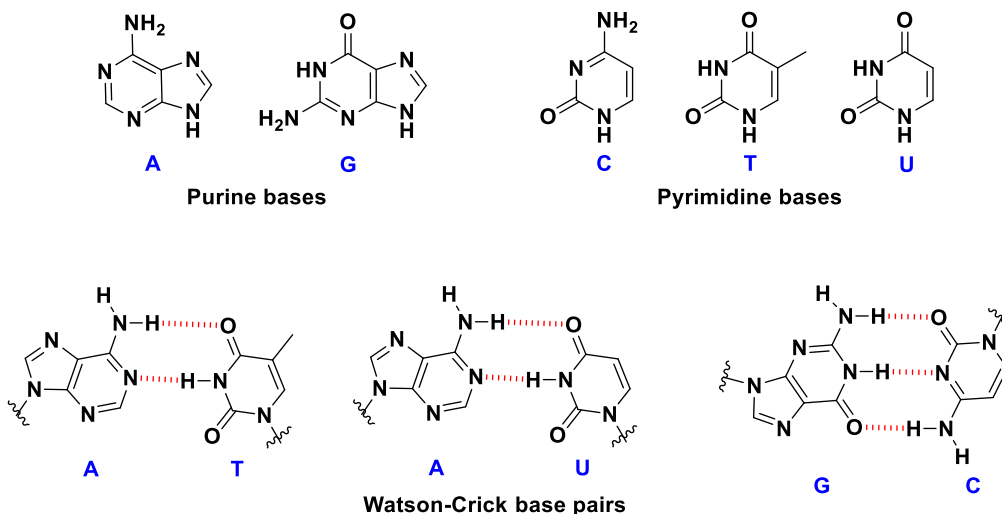


Figure 8. Purine and pyrimidines bases present in nucleic acids and Watson-Crick base pairs.

Protein synthesis is a complex process and ribosomes are the molecular machines that serve as the sites for protein synthesis by a process called translation. Ribosomes connect the amino acids together by peptide bonds in a defined sequence specified by m-RNA. A specific m-RNA bearing the codons for the polypeptide to be made is synthesized in the nucleus from DNA by a process called transcription. Then m-RNA binds to the smaller ribosomal subunit and to the aminoacyl transfer-RNA that carries the amino acid to be incorporated. Finally, the large ribosomal subunit binds to form an initiation complex. Then the polypeptide chain is elongated by covalent attachment of successive amino acids. Each amino acid is carried and correctly positioned by t-RNA. t-RNA contains a complimentary anticodon on one end and amino acid on the other. Based on correct codon-anticodon matching a specific amino acid is transferred to the peptide chain by t-RNA and this process is repeated to form a polypeptide chain. The ribosome contains three RNA binding sites: (i) A-site; aminoacyl t-RNA binding site, (ii) P-site; peptidyl t-RNA (t-RNA bound to the peptide chain being synthesized) binding site, and (iii) E-site; empty t-RNA (before it leaves the ribosomes) binding site (Figure 9).⁴⁴

Figure 9. Schematic representation of protein synthesis.

10).^{18,20,28,45-48}

The neamine core (rings I and II) of both the 4,5- and 4,6-classes of AGAs is mainly responsible for binding. The 6'-substituent and the ring oxygen of the neamine core (e.g., NH₂ in neomycin and OH in paromomycin) interact through hydrogen bonding with N-1 and N-6 of A-1408 and making a pseudo base-pair type interaction. Binding of the neamine core is also characterized by CH- π interaction of the β -face of ring-I with G-1491. The 2-Deoxystreptamine ring (ring-II) of the neamine core forms hydrogen bonds with G-1494 and U-1495. Rings III and IV of the 4,5-series of AGAs reaches to the base pairs 1409-1491 and 1410-1490, which gives additional hydrogen bonding interactions between the 5''-hydroxyl group and N-7 of G-1491 (Figure 12). In the case of the 4,6-series of AGAs, ring-III is located in a different position and forms hydrogen bond to G-1405.^{49,50} This mode of AGA binding causes residues A-1492 and A-1493 to adopt a flipped out conformation in which they interact with minor groove of the codon-

anticodon helix (Figure 13).^{18,51-53} This flipped out conformation stabilizes the t-RNA-A-site complex, facilitates misreading, and increases the energy barrier for translocation.²²

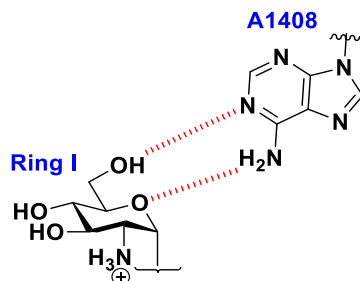


Figure 11. Pseudo base-pair type interaction between ring-I of paromomycin and residue A-1408.

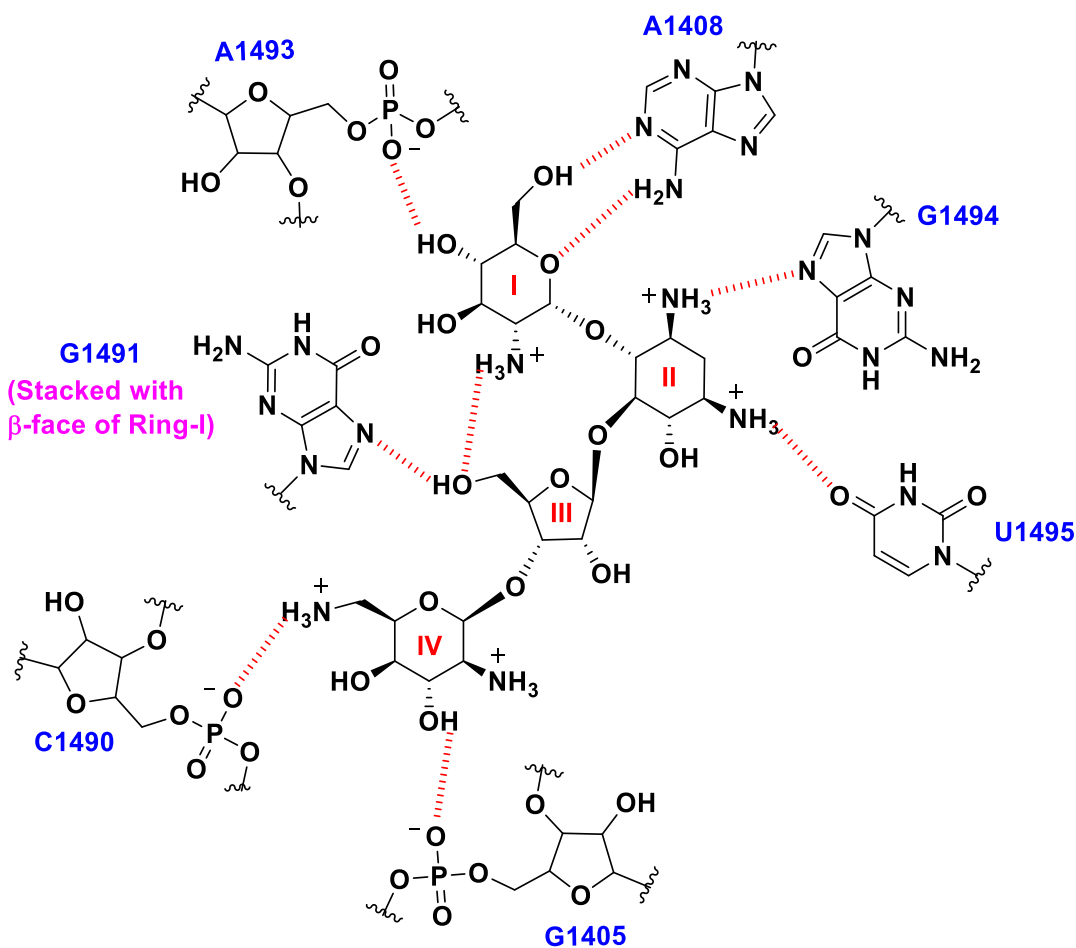


Figure 12. Schematic representation of paromomycin (4,5-series AGA) interactions with bacterial 16S r-RNA.

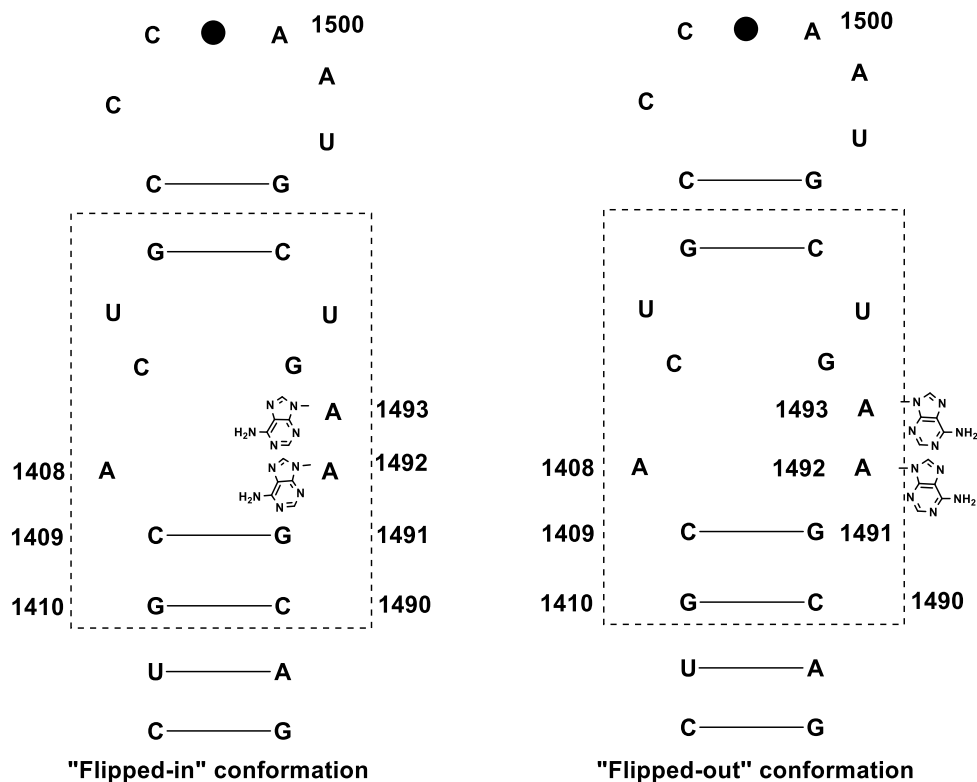


Figure 13. Schematic representation “flipped-in” and “flipped-out” conformation of adenines in bacterial A-site.

1.4. Toxicity and selectivity of AGAs:

The therapeutic use of AGAs and their development is inhibited by their toxicity. The main toxic responses are nephrotoxicity (kidney damage) and ototoxicity (hearing damage).^{25,54-56}

1.4.1. Nephrotoxicity:

Nephrotoxicity is one of the well-known side-effects associated with AGAs and it is a drug-induced kidney damage that leads to their inability to eliminate urine and other wastes from the body. The reported incidence of AGA-induced nephrotoxicity fluctuates between 5 and 26 %, depending on various factors. AGAs are largely eliminated from the body through glomerular filtration by kidneys and excretion in the urine. After glomerular filtration, a significant accumulation (~5 % of the administered dose) of AGAs in the epithelial cells lining S1 and S2

segments of the proximal tubules is the main cause of nephrotoxicity. Because of their polycationic nature at physiological pH, AGAs show a high affinity for phospholipid receptors on the brush border cell of the proximal convoluted tubule and pars recta. Subsequently, the AGAs are reabsorbed by a process called pinocytosis and accumulate in lysosomes and other subcellular compartments of the proximal tubular cell. Ultimately proximal tubular cell damage occurs and leads to nephrotoxicity.

Nephrotoxicity is reversible and rarely leads to a fatal outcome, nevertheless it is desirable to minimize it. In the clinic, this is achieved by injection of a single large daily dose rather than several small doses. This method of injection was developed on the basis of the fact that the AGA uptake by kidney tubular cells is saturable, meaning the uptake of AGAs by the kidneys is limited even when large doses are given.^{57,58} Additionally, hydration treatments can alleviate the symptoms of AGA-induced nephrotoxicity.²⁸ Also, co-administration of polyaspartic acid with AGAs has been shown to prevent their binding to phospholipids, thereby reducing the AGA-induced nephrotoxicity.⁵⁹

1.4.2. Ototoxicity:

Streptomycin and other AGAs target sensory hair cells of the inner ear and can lead to hair cell degeneration and permanent loss. This ototoxicity is the most important and severe toxic response of AGAs. Ototoxicity is irreversible and is reported to affect up to 20% of the patient population on extended treatment.²⁵ There are two types of ototoxicity: (i) vestibular toxicity, and (ii) cochlear toxicity. AGAs destroy the vestibular and cochlear cells by inhibiting the mitochondrial protein synthesis which ultimately leads to hearing loss. The permanence of AGA-induced toxicity is the result of degeneration of hair cells in the cochlea, which cells don't regenerate once damaged.^{28,60-62} The magnitude of ototoxicity is variable and influenced by several

factors. One factor is the type of AGA used since different AGAs have different toxic potentials. For instance, neomycin is more ototoxic than gentamicin or tobramycin, which in turn more ototoxic than netilmicin. Another factor is the dose and duration of treatment. Longer usage of AGAs, as in the treatment of tuberculosis, leads to a higher incidence of ototoxicity. Impaired kidney functions and nutritional or physiological conditions of the patient may also affect the magnitude of ototoxicity.⁶³

AGAs enter the inner ear quickly within few minutes of administration. The half-life of most AGAs in serum is 3-5 h, but their half-life in the inner ear may exceed 30 days.⁶⁴ The primary targets of AGAs are the sensory hair cells of the inner ear, which are essential for the transduction of auditory stimuli (inner and outer hair cells) and balance sensation (type-I and type-II vestibular hair cells).^{65,66} The AGAs carry a positive charge on protonated amino group under physiological pH, which causes a number of actions and enable them to bind with negatively charged cell components or displace cations from binding sites. Recent studies suggest that ototoxicity is due to the inhibition of human mitochondrial protein synthesis or the promotion of abnormal mitochondrial protein synthesis leading to ROS formation and ultimately cell death, arising from binding between AGAs and the human mitochondrial A-site. The mechanism of ototoxicity is therefore related to the mechanism of bactericidal activity.^{48,67-69} Ototoxicity occurs in two ways: (i) a sporadic dose-dependent manner in general patients; (ii) an aggravated manner in genetically susceptible people. This susceptibility is linked to a mutation in mitochondrial r-RNA. In particular, when the A1555 residue is mutated to G in the A-site of mitoribosomal small subunit (Figure 10).⁷⁰

Many efforts have been made to attenuate ototoxicity in animals, for example, by co-administration of various vitamins, amino acids, hormones, antibiotics, sulfhydryl compounds, etc., but none have been developed into a successful clinical treatment for the prevention of AGA-induced ototoxicity. However, antioxidant therapy to overcome the effects of ROS formation is considered a promising lead for the mitigation of ototoxicity. Antioxidants have been found effective against both the vestibular and cochlear ototoxicity.^{71,72} For example, salicylate, the active ingredient of aspirin, which act as both an antioxidant and iron-chelator has been shown to reduce gentamicin-induced ototoxicity.⁷³

1.4.3. Selectivity of AGAs:

The small differences between eukaryotic and prokaryotic ribosomes can be exploited for the development of new generation of AGAs. AGAs bind more tightly to the bacterial ribosomal A-site than the human ribosomal A-site. The human cytosolic A-site differs from bacterial A-site in three ways. The first difference is in position 1408 which is an A residue in the bacterial A-site but is a G in the cytosolic A-site. This change disrupts the key hydrogen bonding interaction to the 6'-substituent in the neamine core. The second difference is in the 1409-1491 base pair (C-G in bacteria, but C-A in human cytosolic A-site), and the third is in the 1410-1490 base pair (G-C in bacteria, but U-A in human cytosolic A-site).^{50,69} These changes are sufficient to cause selective binding of the AGAs to the bacterial ribosome over the human cytosolic ribosome, meaning that systemic toxicity is not a major consideration. The human mitochondrial A-site on the other hand retains the critical A-1408 residue of the bacterial A-site, but differs in the positions 1409-1491 (C-G in bacteria, but C-C in human mitochondrial A-site) and 1410-1490 (G-C in bacteria, but C-A in human mitochondrial A-site) (Figure 14). Consequently, AGAs show lower selectivity for the bacteria A-site over the mitochondrial A-site, resulting in ototoxicity.^{69,74} The single nucleotide

mutation A1555G in the mitochondrial A-site increases the similarity to the bacterial A-site, resulting in tighter AGA binding, and causing susceptibility to AGA-induced ototoxicity in affected people (Figure 15).^{69,74,75}

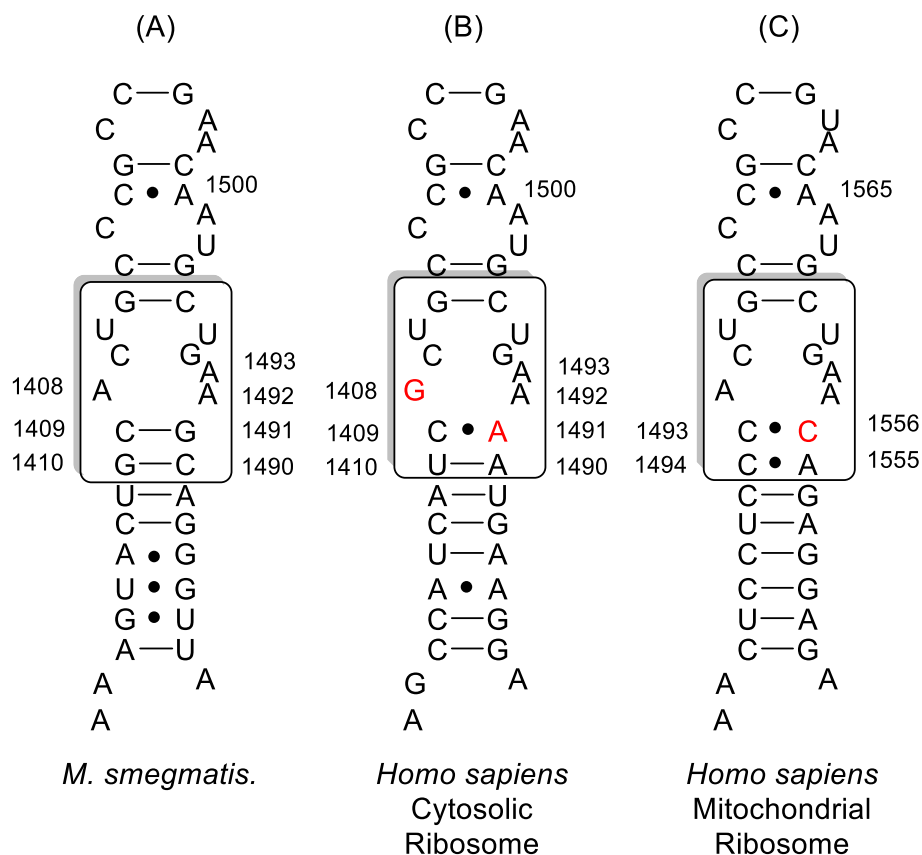


Figure 14. Origin dependent differences in the r-RNA decoding A-Site (AGA binding site is shown in the box).

A central hypothesis of this thesis is that the differences between the bacterial and mitochondrial A-sites can be exploited in the design of improved and more selective AGAs presenting reduced ototoxicity. Prof. Erick Böttger's lab in the University of Zurich screen the newly synthesized AGA derivatives for their antibacterial properties and selectivity. The ribosomal drug susceptibility was analyzed on a single r-RNA allelic derivative of the eubacterium *Mycobacterium smegmatis*^{48,76} and it was determined by establishing the Minimal Inhibitory

Concentration values (MIC values) for the growth of *M. smegmatis*. The MIC value is the lowest concentration of the AGAs, which completely inhibit the growth of bacteria. Prof. Böttger and his coworkers developed cell free translation assays with purified 70S bacterial ribosome of both wild type and recombinant hybrid ^{45,77} carrying the complete decoding A-site cassettes of human mitochondrial (Mit 13), the A1555G mutant of the human mitochondrial (A1555G), and the human cytosolic (Cyt 14) ribosomes (Figure 14). By the help of these translation assays, IC₅₀ values were determined for each synthesized AGA. The IC₅₀ value is the concentration of AGA required to inhibit the protein synthesis by 50 percent.

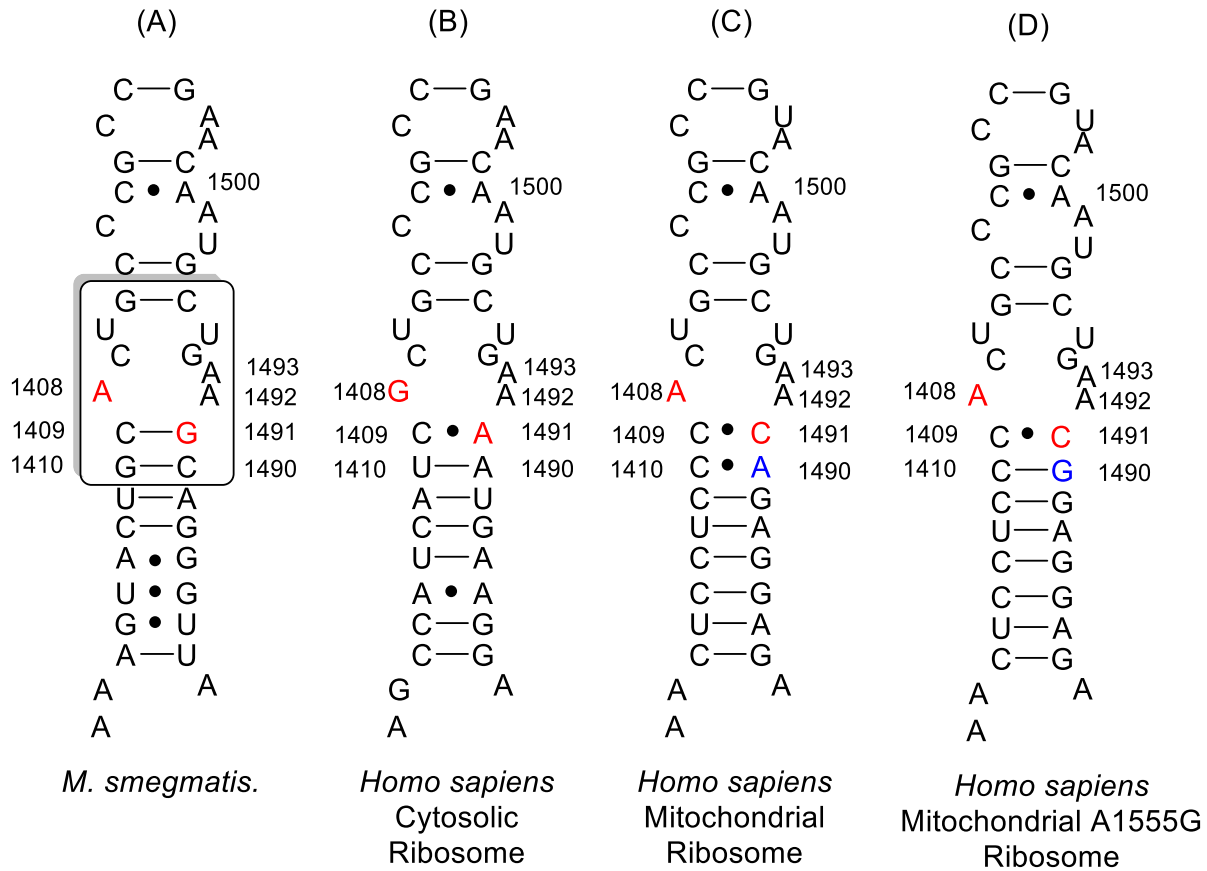


Figure 15. Origin dependent differences in the r-RNA decoding A-Site (Bacterial numbering is used throughout for clarity and A1555 in the mitochondrial numbering corresponds to A1490 in the bacterial numbering).

1.5. Bacterial resistance to AGAs:

Circumventing antibiotic resistance is an important factor in the development of novel AGAs as therapeutic agents. Aminoglycoside resistance occurs through several mechanisms but the four are: (i) decreased uptake of the AGAs in bacteria; (ii) AGA efflux; (iii) target modification; and (iv) aminoglycoside modifying enzymes.

1.5.1. Decreased uptake of AGAs:

Reduced drug uptake is due to membrane impermeabilization or diminished inner membrane transport. The bacterial cell wall is the natural barrier for small molecules such as AGAs and for their action they must transverse the cell wall. AGAs carry a positive charge at physiological pH and have a very hydrophilic nature. Accordingly, it is hypothesized that they cross the bacterial cell wall through porin channels rather than by direct diffusion through the phospholipid bilayer.^{78,79} The sugar-modified phospholipids (LPSs) present on the outer membrane bear a net negative charge and attract the cationic AGAs. The most common LPS modification is the incorporation of 4-amino-4-deoxy-L-arabinose, which reduces the net negative charge of the LPS layer and causes the decreased AGA-uptake.⁸⁰⁻⁸² Additionally, phosphoethanolamine was also reported to be incorporated in response to the presence of cationic molecules.⁸³ The lipid composition of innermost layer of the inner membrane in *Mycobacterium smegmatis* has been reported to consist of an unusual lipid, diacylphosphatidylinositol dimannoside.⁸⁴ This lipid has four hydrocarbon chains and is proposed to lead to poor drug permeability. In some bacteria like *E. coli*, *S. aureus*, and *P. aeruginosa*, mutations in ATP-synthase also resulted in their decreased susceptibility to AGAs.⁸⁵

The porin proteins considered to be the point of entry of the AGAs are water filled channels allowing passive diffusion of hydrophilic small molecules. Ompf is the classical porin in *E. coli*

which transports small molecules, but there is no conclusive evidence available to support its role in AGA transport.⁸⁶ Two type of porins have been reported in mycobacteria: MspA-like from *M. Smegmatis* and OmpA-like from *M. tuberculosis*. Many studies on MspA-like porins have shown that deletion of porins results in reduced drug uptake.⁸⁷ However, for kanamycin the Msp deletion did not cause a significant increase in MIC values, suggesting that these porins are not directly involved in AGA-transportation.⁸⁸ The crystal structure of MspA also suggest that it is not directly involved in AGA resistance.⁸⁹ These observations indicate the need for better understanding of AGA-uptake mechanism.

1.5.2. AGA efflux:

AGA efflux is another mechanism of AGA-resistance which involve active transport of AGAs out of the cells by efflux pumps. There are five known classes of efflux pumps, of which the resistance nodulation division (RND) is the most widespread family in Gram-negative bacteria.⁹⁰ These RND efflux pumps consist of three main components: the RND pump, a periplasmic membrane fusion protein (MFP), and an outer-membrane factor (OMF). Together these three proteins effectively pump a variety of antibiotics, dyes, and ions out of the cells. AGAs are known to be poor substrates for some other efflux pumps. For example, the small multidrug resistance (SMR) transporter in *P. aeruginosa*,⁹¹ the multidrug ABC transporter in *L. lactis*,⁹² and the major facilitator superfamily (MFS) in *E. coli*.⁹⁰ The multidrug and toxic compound extrusion (MATE) family of efflux pumps have also been shown to transport AGAs in *V. cholerea*.⁹³ These energy-dependent bacterial efflux pumps lower the AGA concentration in the bacterial cell and now identified as one of the major cause of antibiotic resistance.

1.5.3. Target modification:

AGA target the 16S r-RNA present in the A-site of bacterial ribosome and in this way, they inhibit the bacterial protein synthesis leading to the cell death. Therefore, any modification in the binding site will significantly affect the antibacterial activity of AGAs. There are two most common target modification mechanism known to cause AGA-resistance in several bacteria: mutation in ribosomal A-site and enzymatic modification of ribosomal A-site.

1.5.3.1. Mutations in the ribosomal A-site:

AGA resistance may arise from mutations in the *rrs* gene, which codes for 16S r-RNA and causes decreased binding of AGAs to 16S r-RNA.⁹⁴ The most common example is a A1408G mutation, which causes a high level of resistance for gentamicin, tobramycin, kanamycin, amikacin, arbekacin, isepamicin and neomycin.^{95,96} The non-DOS AGA streptomycin which interacts with the ribosomal protein S12 in addition to 16S r-RNA, is also rendered ineffective by this mechanism. Thus, mutations in either the 16S r-RNA or the S12 protein, result in high levels of resistance to streptomycin in *M. tuberculosis*.^{97,98} Similarly, *N. gonorrhoeae* has been shown to contain a mutation in ribosomal protein S5, which causes resistance to spectinomycin.⁹⁹

1.5.3.2. Enzymatic modification of the ribosomal A-site:

A significant amount of resistance is caused by enzymatic methylation of certain bases in the ribosomal A-site. These methylations are catalyzed by the enzymes commonly referred as RNA methyltransferases.¹⁰⁰ These methyltransferases naturally occur in AGA-producing bacteria (*Streptomyces* and *Micromonospora*) where they protect the parent organism from the AGAs they produce by methylation of 16S r-RNA.^{101,102} For example, *M. purpurea* (gentamicin producer) and *S. tenebrarius* (tobramycin producer) encode Sadenosylmethionine-dependent methyltransferases, which modify G1405 or A1408 to the 7-methyl derivatives.¹⁰³ Previously these methyltransferases

were confined to the non-pathogenic actinomycetes and have only recently been reported to cause resistance to AGAs in various pathogenic bacteria. The common examples are, RmtA in *P. aeruginosa*;¹⁰⁴ RmtB in *S. marcescens*, *A. baumannii*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*;¹⁰⁵ RmtC in *K. pneumoniae*;¹⁰⁶ and Arm in *S. marcescens*, *E. coli*, and *K. pneumoniae*.^{101,107,108} The modifications caused by methyltransferases pose a serious threat to the 4,6-series of AGAs including plazomicin, as they mostly interfere with the G1405 residue that contacts ring-III of that series.¹⁰⁹⁻¹¹²

1.5.4. Aminoglycoside modifying enzymes (AMEs):

AMEs are the most widespread and clinically relevant mechanism of AGA-resistance. AMEs are bacterially expressed enzymes which modify the antibiotics and thereby inactivate them.²³⁻²⁷ These enzymes catalyze the covalent modification of amino or hydroxyl groups in AGAs thereby disrupting AGA binding to the ribosome and leading to compounds devoid of antibiotic activity. AMEs can be classified into three major categories: (i) Aminoglycoside *N*-acetyltransferases (AACs), (ii) Aminoglycoside *O*-phosphotransferases (APHs), and (iii) Aminoglycoside *O*-nucleotidyltransferases (ANTs). AACs use acetyl coenzyme-A as a donor and modify the amino groups. Both ANT and APHs use ATP as a donor and affect the hydroxyl functions. Figure 15 depicts the potential modification sites of some representative AGAs.

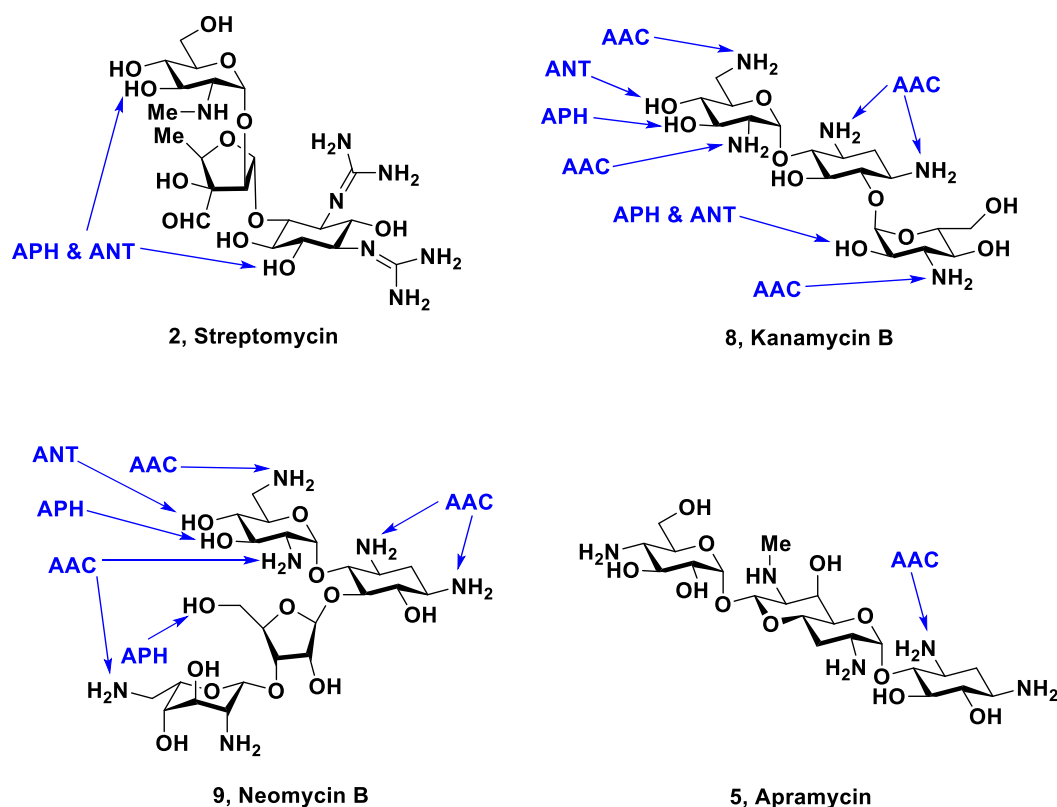


Figure 16. Potential modification sites of streptomycin, kanamycin B, neomycin B, and apramycin.

1.5.4.1. Aminoglycoside *N*-acetyltransferases (AACs):

These enzymes act by acetylating one or more amino groups in the AGAs. AACs use acetyl-CoA to transfer the acetyl functionality. By acetylating the amino groups, they (i) reduce the positive charge on AGAs and (ii) result in a steric block to interaction with residues in the binding site. There are four major categories of AACs based on their regiospecificity for acetyl transfer: AAC (6'), AAC (2'), AAC (1), and AAC (3). In this notation, the number in parentheses indicates the specific positions the enzyme acetylates AGAs. Four AAC enzymes have been crystallized and their 3D structures determined; these are AAC (3)-Ia from *Serratia marcesans*,¹¹³ AAC (6')-Ii from *Enterococcus faecium*,¹¹⁴ AAC (6')-Iy from *Salmonella enterica*,¹¹⁵ and AAC (2')-Ic from *Mycobacterium tuberculosis*.¹¹⁶ Their structures revealed that they are closely related with the

GCN5-related *N*-acetyltransferases (GNAT) protein superfamily.¹¹⁷ Now more than 50 different AAC enzymes have been identified and subcategorized based on their regiospecificity.²⁸ These enzymes are present in both Gram-positive and Gram-negative and confer a broad aminoglycoside resistance profile. Recently there are reports of the emergence of a bifunctional enzyme AAC (6')-APH (2''), which can catalyze both acetylation and phosphorylation in the substrate AGAs. This enzyme can modify almost all the DOS based AGAs and is of serious concern.^{118,119}

1.5.4.2. Aminoglycoside *O*-phosphotransferases (APHs):

These enzymes phosphorylate the key hydroxyl functionalities on AGAs using ATP as a donor. The phosphorylation of hydroxy groups blocks their hydrogen bond donor ability and therefore disrupts key hydrogen bond interactions of AGAs with the bacterial A-site. In addition, phosphorylation introduces a negative charge on AGAs, which reduces their affinity to the bacterial A-site.^{120,121} Seven classes of APH enzyme have been identified at present; APH-(3'), APH-(2''), APH-(3''), APH-(4), APH-(7''), APH-(6), and APH-(9) have been identified till now. APH-(3') is the most widespread and well-studied enzyme; it is generally found in gram-positive bacteria.¹³ There are eight main types of enzymes, from APH-(3')-I to APH-(3')-VIII have been identified in APH-(3') sub-class. The APH-(III)a is the most extensively studied enzyme and known to catalyze phosphorylation of a wide range of AGAs.¹²² Several extensive studies have been performed on regioselectivity of phosphorylation by APH-(3')-IIIa and it was proposed that the 4,5-disubstituted AGAs lacking a 3'-hydroxy group (e.g. lividomycin A), could also be phosphorylated at 5''-position.¹²³ The APH-(3')-IIIa bound conformations of amikacin, a 4,6-disubstituted AGA, and butirosin A, a 4,5-disubstituted AGA, were probed and it was found that their binding pattern is different. In case of amikacin, only 3'-hydroxy group approaches the γ -phosphate of ATP, whereas in case of butirosin A, both the 3'- and 5''-hydroxyl groups approach

the γ -phosphate of ATP.¹²⁴ These results were further confirmed by showing the exclusive monophosphorylation in 4,6-series of AGAs and mono as well as bisphosphorylation in the case of 4,5-series of AGAs.¹²⁵ Another member which has the structural information is APH-(2'') and it favors 4,6-AGAs over 4,5-AGAs.¹²⁶

1.5.4.3. Aminoglycoside *O*-nucleotidyltransferases (ANTs):

The third mechanism of AGA modification is the ATP-based transfer of AMP group on hydroxyl functionalities by the enzymes called ANTs. There are five classes of ANT enzymes based on their regiospecificity, ANT-(2''), ANT-(4'), ANT-(3''), ANT-(6'), and ANT-(9). These are the smallest family of AMEs with only ten enzymes identified till now.²⁸ ANT-(2'') is the most clinically significant class and widespread among gram-negative bacteria. ANT-(2'') caused a significant amount of resistance to gentamicin, tobramycin, and amikacin (Figure 17).

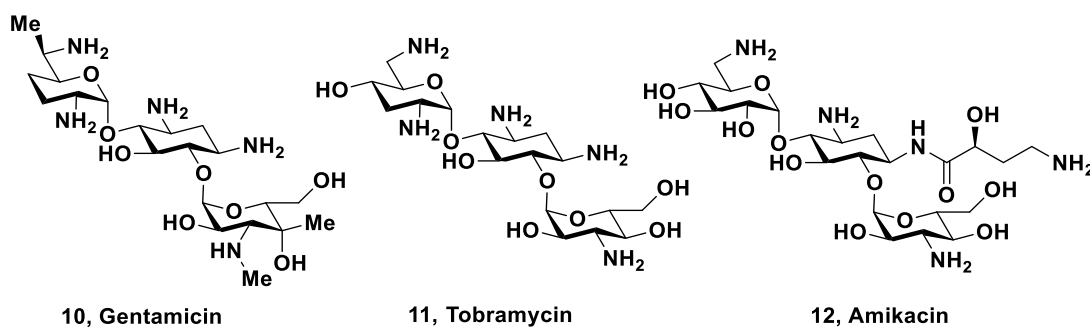


Figure 17. Structures of gentamicin, tobramycin, and amikacin.

1.5.5. Strategies to overcome aminoglycoside resistance:

AGAs are the highly potent broad-spectrum antibiotics and they have much desired bactericidal activity. Their clinical use is somewhat limited by the emergence of resistant bacterial strains. As a result, there is no new AGA introduced in the clinics for a number of years. But with the emergence of resistance to other class of antibiotics, the availability of detailed atomic level understanding of AGA binding to the bacterial A-site and well-studied mechanism of their

resistance have offered an opportunity to revisit the AGAs and design the new more efficient antibiotics of this class. Commonly two approaches have been employed to avoid the resistance: inhibition of resistance and resistance-proof AGAs.

A small molecule inhibitor of AMEs could be a good strategy to avoid the resistance and this strategy has been successful for β -lactam antibiotics.¹²⁷ 7-Hydroxytropolone **13**, was reported to have inhibitory activity on ANT-(2'').¹²⁸ Similarly, the compounds with the structure like **14**, have been reported to have inhibitory activity on AAC-(6').¹²⁹ Based on the fact that AMEs have a negatively charged binding pocket to accommodate cationic AGA, many cationic peptides were screened against several AMEs, and their inhibitory activity against several enzymes were identified.¹³⁰

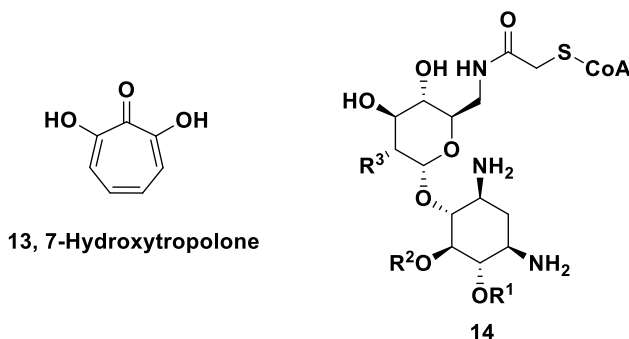


Figure 18. Some of the AME inhibitors.

The other and better approach is to rationally design the resistance-proof AGAs those can avoid the modifications by AMEs. Tobramycin and gentamicin don't have the 3'-hydroxyl group and are not susceptible to APH-(3'). Amikacin, a semisynthetic derivative of kanamycin has a better activity against many AGA resistant strains. Neamine dimers having a structure like **15**, are reported not to be affected by AAC-(6'), APH-(3'), and APH-(2'').¹³¹ Semisynthetic derivatives of neamine, termed as pyranmycin **16** and pyrankacin **17** retain their activity against resistant strain of *E. Coli*, *K. pneumoniae*, and *S. aureus*.^{132,133} The semisynthetic neamine derivative **18** was

designed to mimic amikacin and was found to be a poor substrate for modification by APH-(3'), AAC-(6') and APH-(2'').^{20,134} Conformationally rigid neomycin B derivative **19** was designed to resemble the confirmation of neomycin B bound to bacterial A-site, but to differ from the confirmation of neomycin bound to ANT-(4') active site. As desired this compound is a poor substrate for modification by ANT-(4') and AAC-(2').¹³⁵ Additionally, AGA hybrids having a structure like **20**, which contain features of neomycin B or paromomycin with sisomicin were shown to display good antibacterial and to evade APH-(3') and ANT-(4') resistance mechanisms.¹³⁶ These results are very promising and show that it is possible to build new generation of resistance-proof AGAs (Figure 19).

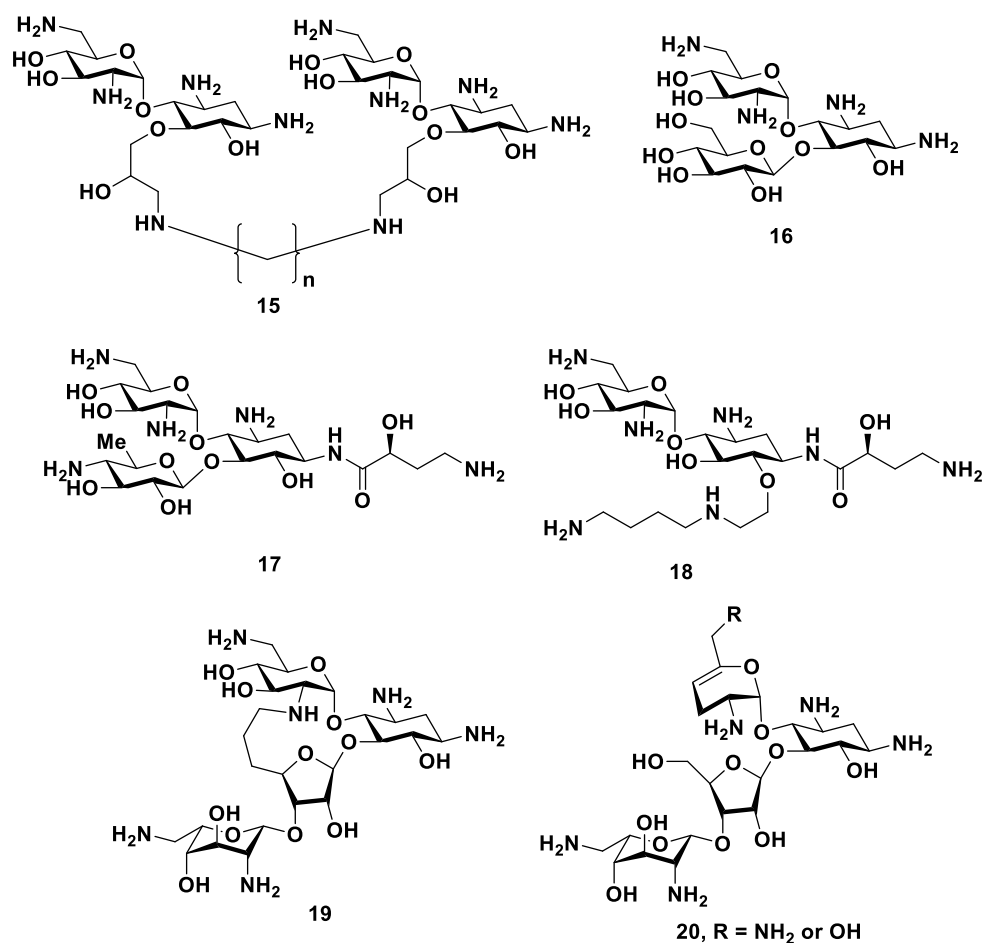


Figure 19. Some of the rationally designed semisynthetic AGAs.

1.6. Goals:

The overall goal of this research project is to rationally design and synthesize novel aminoglycoside antibiotics which will cause little to no ototoxicity and completely evade the modifications by common AMEs. The differences between the bacterial and mitochondrial A-sites can be exploited in the design of improved and more selective AGAs presenting reduced ototoxicity. At the same time, well understood mechanism of resistance to AGA can be used to design new AGAs which are active against resistant strains of various bacteria. For this purpose, Neomycin B and paromomycin from 4,5-series of AGAs were selected. Neomycin B and paromomycin are highly potent broad spectrum antibiotics and discovered almost six decades ago. Their clinical use was limited due to their toxic responses and evolution of resistant bacteria. They are one of the most extensively studied aminoglycoside antibiotics. Their binding pattern to bacterial and mitochondrial A-sites is well known and mechanism of resistance is also well studied. These earlier observations reported in the literature on neomycin B and paromomycin made them ideal substrates for further modifications and to determine the influence of these modifications on antibacterial activity, ribosomal selectivity, and activity against resistant strains of bacteria to determine their susceptibility against common AMEs. In neomycin B series, the 2', 4', 6', and 6'''-positions will be targeted for modifications. Similarly, in paromomycin will be modified at 2' and 5''-positions. Based on the information obtained from these single modifications, advanced compound will be made with multiple modifications.

CHAPTER 2. MODIFICATIONS TO NEOMYCIN B.

2.1. *N*6'-, *N*6'''-, and *O*4'-modifications to neomycin B.¹³⁷

2.1.1. Introduction:

Neomycin B is a 4,6-series AGA that was discovered by Selman Waksman in 1949. It is naturally produced by the bacterium *Streptomyces fradiae*. Neomycin B is a very potent broad spectrum antibiotic and its binding pattern to bacterial ribosome is similar to paromomycin shown in Figure 11. The only difference between neomycin B and paromomycin is the 6'-substituent; an amino group in neomycin B but a hydroxyl group in paromomycin. This 6'-amino present in neomycin B increases its affinity to bacterial ribosome and responsible for its higher antibacterial activity as compared to the paromomycin. This 6-amino group also results in higher affinity of neomycin B for human mitochondrial ribosome and its A1555G mutant and makes it more ototoxic as compared to the paromomycin.^{55,56,70,74,75,138} This can be explained based on the fact that hydrogen bond involving an ammonium ion as a donor in the case of neomycin B is estimated to be ≥ 3 kcal.mol⁻¹ stronger than the hydrogen bond involving a hydroxy group as a donor in the case of paromomycin.⁶⁷ Additionally, 6-amino group makes neomycin B susceptible to inactivation by the AAC-(6') class of AMEs.^{110,139} In recent years, many reports have been published on the synthesis of improved neomycin B derivatives, including 6'-*N*-alkyl derivatives with reduced susceptibility to AAC-(6').¹⁴⁰⁻¹⁴⁸ It has also been reported that 4'-*O*-ethylation of paromomycin increases its selectivity for the bacterial over the human cytosolic and mitochondrial ribosomes and decreases the susceptibility to inactivation by AMEs.¹⁴⁹ Based on these observations reported in the past, neomycin B was modified either at 6'-position alone and in combination with 4'-*O*-ethylation. The 2-hydroxyethyl group was selected for 6'-*N*-alkylation. It was first introduced in the sisomicin series^{150,151} and also present in plazomicin (Figure 20).³⁶

These 1,2- and 1,3-amino alcohol modifications do not cause reduction in antibacterial activity of AGAs and suggested to enhance binding to phosphate backbone and nucleic acid bases.^{152,153} Moreover, the presence of 2-hydroxyethyl group would decrease the basicity of the amino group¹⁵⁴ and would reduce the affinity of neomycin B for the human mitochondrial ribosome and its A1555G mutant. The synthetic routes also provided easy access to the other neomycin derivatives modified at 5''- and 6'''-positions and they were also screened for their antiribosomal and antibacterial activities.

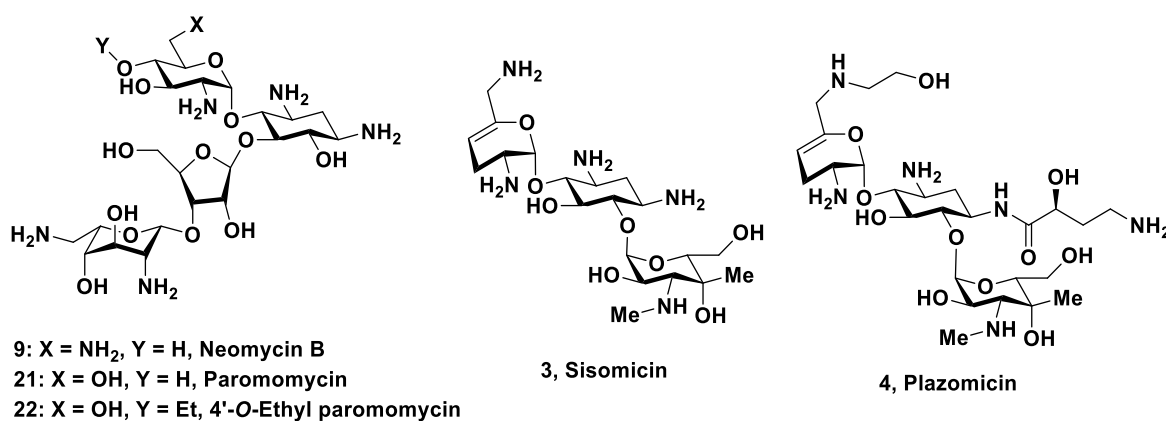


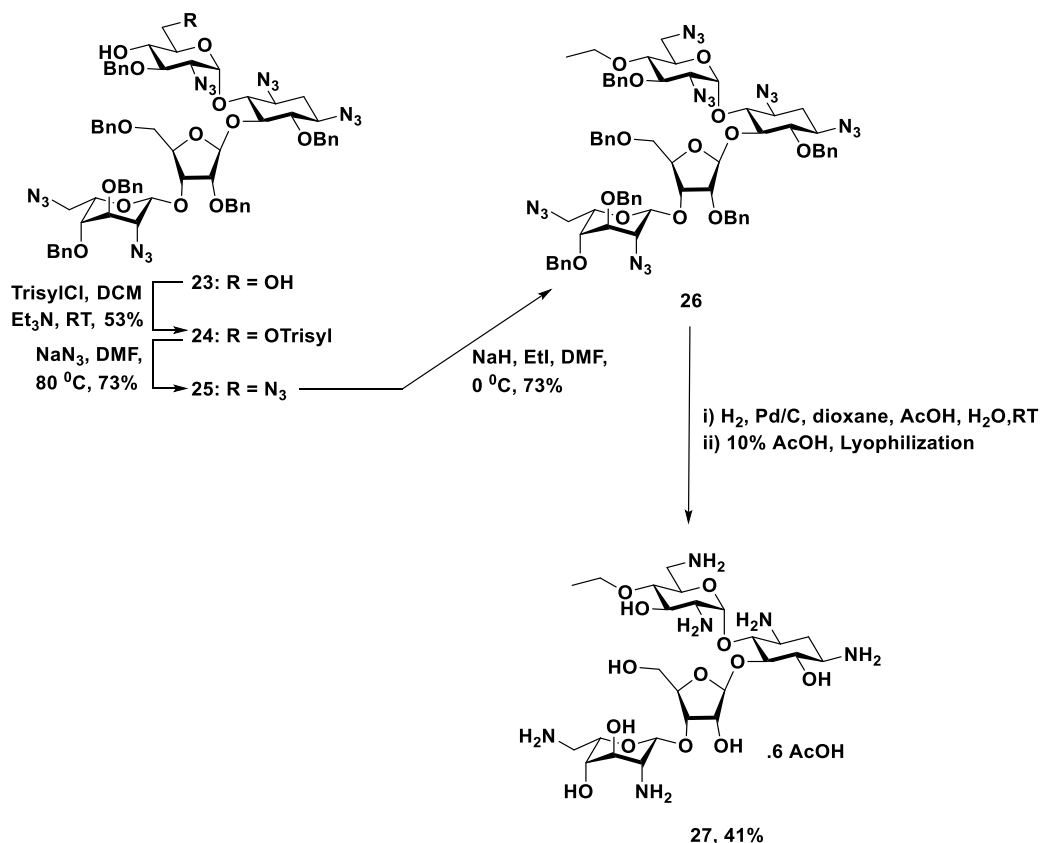
Figure 20. Structures of neomycin B, paromomycin, 4'-O-ethyl paromomycin, sisomicin, and plazomicin.

2.1.2. Synthesis:

The synthesis of 4'-O-Ethyl neomycin B **27** started from the 4', 6'-diol intermediate **23**, which was readily prepared from paromomycin **21** by following the procedure described in the literature.¹⁵⁵ Selective sulfonylation of **23** with 2,4,6-triisopropylbenzenesulfonyl chloride gave **24** in 53% yield and then displacement with azide afforded intermediate **25** in 73% yield. Ethylation of **25** with sodium hydride and ethyl iodide in *N,N*-dimethylformamide gave intermediate **26** in 73% yield. Global deprotection by hydrogenolysis, followed by purification on Sephadex and

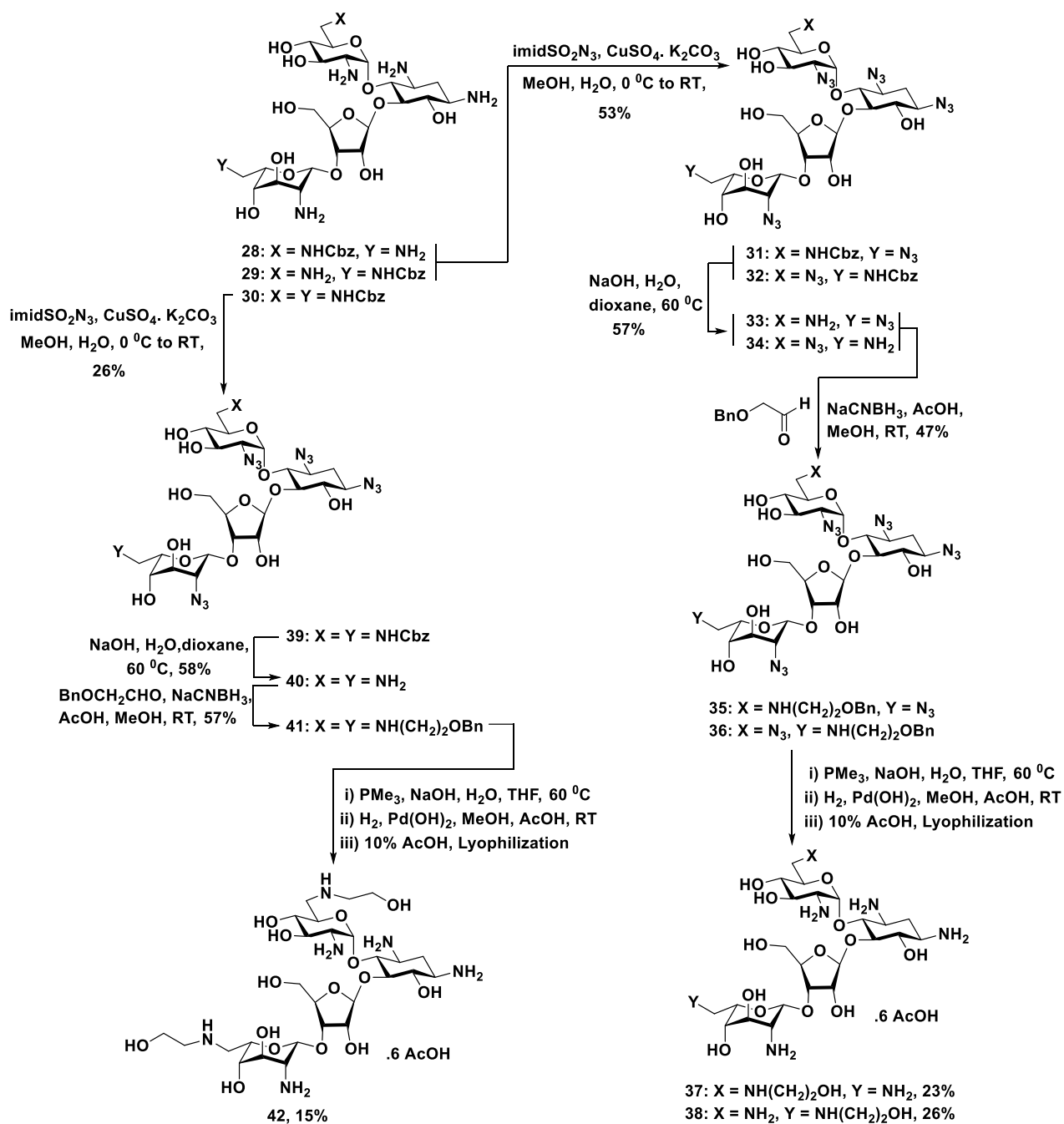
lyophilization from aqueous acetic acid gave **27** as the peracetate salt with a 41% yield (Scheme 1).

The synthesis of 6'-*N*-(2-hydroxyethyl) neomycin B **37** started with the selective protection of 6'-NH₂ group with benzyl carbamate by following a procedure described in the literature,¹⁵⁶ in which treatment of neomycin B sulfate with *N*-(benzyloxycarbonyloxy) succinimide and sodium carbonate in aqueous dioxane gave an inseparable 1:1 mixture of the *N*6'- and *N*6'''-benzyl carbamates **28** and **29** in 32% yield, along with 29% of the *N*6', *N*6'''-bis (benzyl carbamate) **30**. This **28/29** mixture on subjecting to the diazo transfer reaction using imidazolesulfonyl azide^{157,158} in the presence of potassium carbonate and catalytic copper sulfate in aqueous methanol afforded the regioisomeric mixture of pentaazides **31** and **32** in 53% yield. Saponification of benzyl carbamate in hot aqueous dioxane gave a 1:1 mixture of regioisomeric amines **33** and **34** in 57% yield. Reductive amination of the **33/34** mixture with benzyloxyacetaldehyde using sodiumcyanoborohydride¹⁵⁹ as a reducing agent in methanol gave a 1:1 mixture of the *N*6'- and *N*6'''-(2-benzyloxyethyl) derivatives **35** and **36** in a combined 47% yield. This **35/36** mixture was separated by preparative HPLC to obtain pure samples of **35** and **36**. Finally, application of Staudinger reaction to reduce azides with trimethylphosphine and sodium hydroxide in hot aqueous THF,¹⁵⁵ followed by hydrogenolysis to deprotect benzyl ether, and then purification on sephadex and lyophilization from aqueous acetic acid gave the *N*6'- and *N*6'''-(2-hydroxyethyl) derivatives **37** and **38** as their peracetate salts with 23 and 26% yield respectively (Scheme 2). Application of the same sequence of reactions to the *N*6', *N*6'''-biscarbamate intermediate **30** afforded the *N*6', *N*6'''-bis (2-hydroxyethyl) derivative **42** as the peracetate salt, via the intermediates **39-41** (Scheme 2).

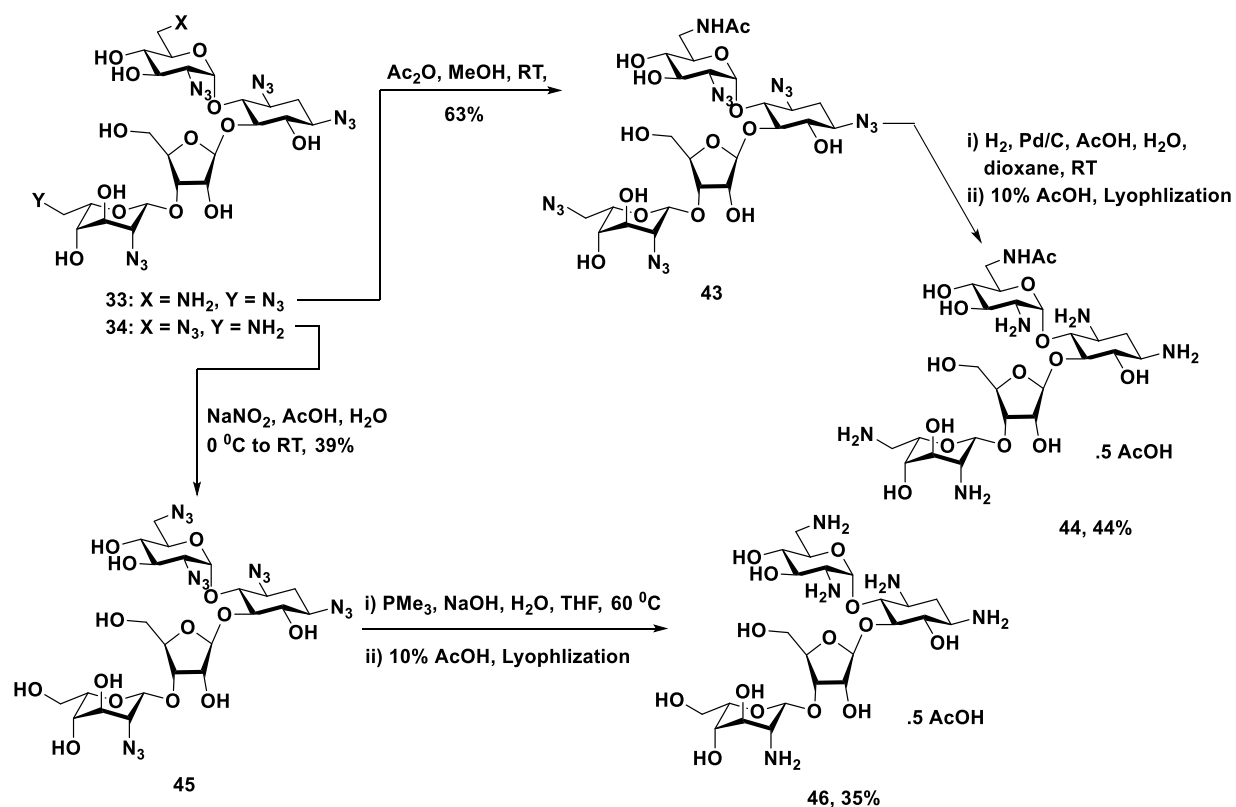


Scheme 1. Synthesis of 4'-*O*-ethyl neomycin B **27**.

Slow and careful separation of the **33/34** mixture in silica gel using a mixture of three solvents (chloroform, methanol, and ammonium hydroxide) was able to give pure samples of the regioisomeric amines **33** and **34**. Treatment of **33** with acetic anhydride in methanol gave the intermediate **43**, which upon reduction of azides by hydrogenolysis, purification on sephedex, and lyophilization from aqueous acetic acid afforded a sample of known 6'-*N*-acetyl neomycin B **44**^{160,161} as peracetate salt in 44% yield (Scheme 3). Diazotization of **34** with sodium nitrite in aqueous acetic acid^{156,162,163} gave the pentazide intermediate **45**¹⁶⁴ in 39% yield. Deprotection of the azides by Staudinger reaction with trimethylphosphine, purification on Sephadex, and lyophilization from aqueous acetic acid afforded the sample of known 6'''-deamino-6'''-hydroxy neomycin B **46**¹⁵⁶ as the peracetate salt in 35% yield (Scheme 3).



Scheme 2. Synthesis of neomycin B derivatives **37**, **38** and **42**.



Scheme 3. Synthesis of neomycin B derivatives **44** and **46**.

The synthesis of doubly modified neomycin B derivative **53** started from 4'-*O*-ethyl paromomycin **22**.¹⁴⁹ Application of the same diazo transfer reaction conditions to 4'-*O*-ethyl paromomycin **22** using imidazolesulfonyl azide in the presence of potassium carbonate and catalytic copper sulfate in aqueous methanol gave the pentaazide intermediate **47** in 57% yield. Treatment of pentaazide **47** with *p*-toluenesulfonyl chloride in pyridine at room temperature gave an approximately 1:0.7 mixture of regioisomeric monotosylates **48** and **49** in a combined 30% yield, along with 10% of the ditosylate **50**. Treatment of **48/49** mixture with ethanolamine at room temperature gave a mixture of 2-hydroxyethyl derivatives **51** and **52** in a combined yield of 52%. This **51/52** mixture was separated by preparative HPLC to obtain pure samples of **51** and **52**. Reduction of the azides by Staudinger reaction with trimethylphosphine, purification on Sephadex, and lyophilization from aqueous acetic acid afforded the neomycin B derivative **53** and

paromomycin derivative **54** as their peracetate salts in 42 and 44% yields respectively (Scheme 4). Application of the same sequence of tosylate displacement by ethanolamine and reduction of azides by Staudinger reaction to the ditosylate intermediate **50** afforded the neomycin B derivative **56** as the peracetate salt via the intermediate **55** (Scheme 4).

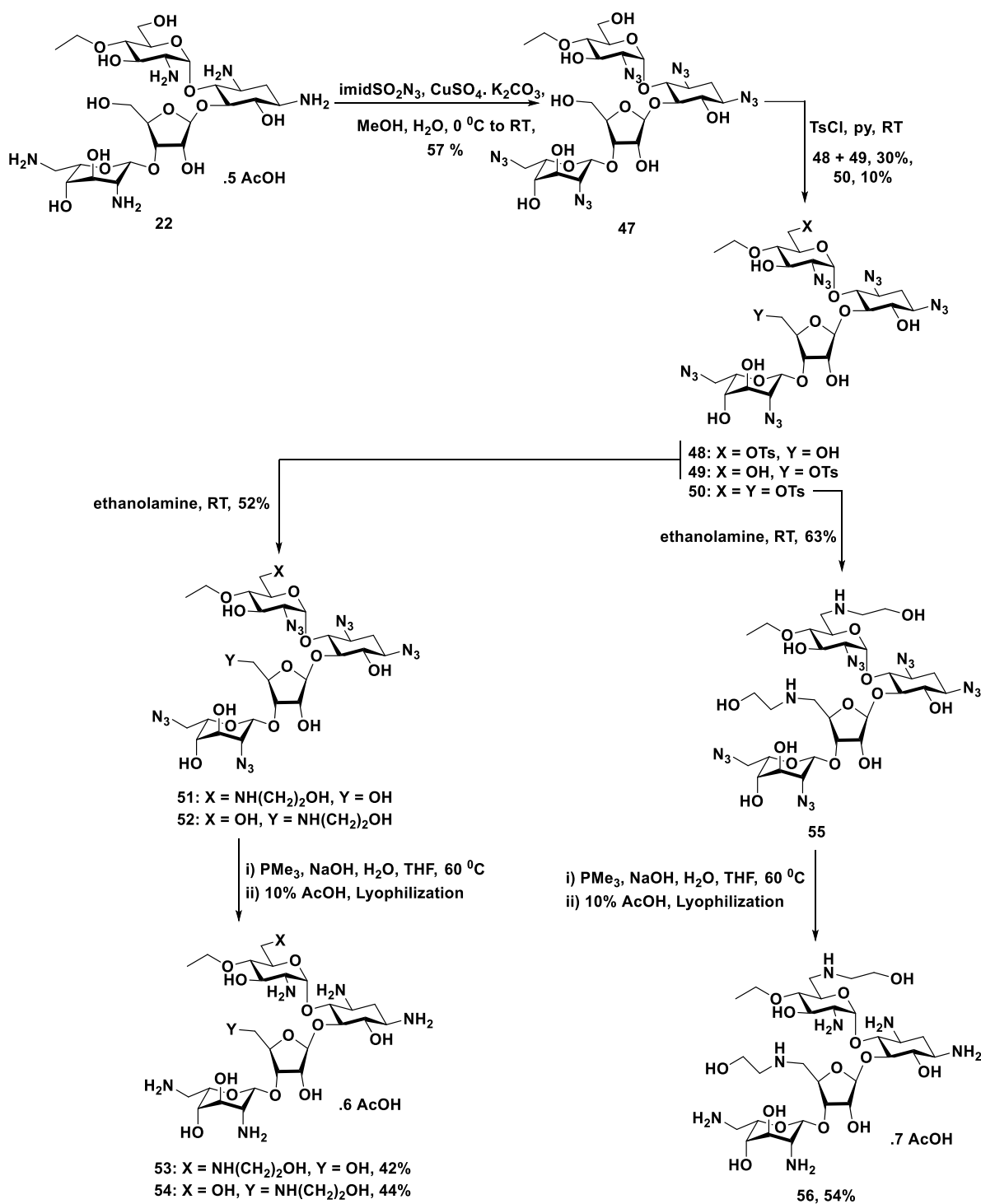
2.1.3. Biological evaluations:

All the synthesized neomycin B derivatives **27**, **37**, **38**, **42**, **44**, **46**, **53**, **54** and **56** were screened to establish their ability to inhibit the protein synthesis. The antiribosomal activities were determined in cell free translational assays employing wild type bacterial ribosomes and recombinant hybrid bacterial ribosomes. Their antiribosomal activities were also compared with the parent neomycin B **9**, paromomycin **21** and 4'-*O*-ethyl paromomycin **22** (Table 1). 4'-*O*-ethylation (**27**) and 6'-*N*-(2-hydroxyethylation) (**37**) do not cause any reduction in antiribosomal activity of neomycin B against the bacterial ribosome. The modifications at 6'''-position also do not have any effect on antiribosomal activity against the bacterial ribosome (**38** and **46**, Table 1). 6'''-*N*-(2-hydroxyethyl) neomycin B **38** and 6'''-deamino-6'''-hydroxy neomycin **46** are as active as the parent neomycin B, which is consistent with the earlier reports on the antibacterial activity of **46**.^{156,165} In contrast, the 4'-*O*-ethylation (**22**) in paromomycin show a 4-fold decrease in antiribosomal activity against bacterial ribosome and similarly the 6'''-deamino-6'''-hydroxy modification also known to cause a significant decrease in antibacterial activity of paromomycin.^{156,165} This difference between the neomycin series and paromomycin series can be explained on the basis of the strength of hydrogen bond between the 6'-substituent and N1 of A1408 residue. The hydrogen bond involving an ammonium ion as a donor in neomycin series is estimated to be ≥ 3 kcal/mol stronger than the hydrogen bond involving a hydroxy group as a donor in paromomycin series.⁶⁷ The 6'-*N*-acetylation (**44**) causes a significant reduction in antiribosomal

activity against bacterial ribosome and it is consistent with the AAC-(6') mechanism of AGA resistance.^{13,14,25,28,30} Double and triple modifications in one single molecule cause a significant reduction in antiribosomal activity against the bacterial ribosome (**42**, **53**, **54** and **56**, Table 1). Therefore, it can be concluded that each of these single modifications have a small detrimental effect on binding, although this small change is not sufficient to be reflected in the IC₅₀ values in antiribosomal assay.

To compare the influence of each modification on ribosomal selectivity, the data presented in Table 1 have been converted into selectivity quotients (SelQ) with respect to the parent neomycin B (Table 2). Paromomycin is 10- to 15-fold more selective for the bacterial over mitochondrial and mutant mitochondrial ribosomes, but less selective over cytosolic ribosome (Entry 1, Table 2). The decrease in selectivity of paromomycin for the bacterial over cytosolic ribosome is due to the fact that the 6'-hydroxyl group of paromomycin can still accept the hydrogen bonds from 1408G residue in cytosolic ribosome in contrast to the repulsive interaction between protonated 6'-amino group of neomycin B and G1408.^{166,167} The 6'-*N*-(2-hydroxyethyl) modification of neomycin B increases selectivity for the bacterial over mutant mitochondrial ribosome to a greater extent than over the wild type mitochondrial ribosome (**37**, Entry 2, Table 2). Similarly, the 6'-*N*-acetyl neomycin B also show a larger increase in selectivity for the bacterial over mutant mitochondrial ribosome as compared to the wild type mitochondrial ribosome (**44**, Entry 5, Table 2). Most probably, the looser wild type mitochondrial A-site accommodates the 6'-*N*-(2-hydroxyethyl) and 6'-*N*-acetyl group better than the more rigid mutant mitochondrial A-site. The slight increase in selectivity of **37** for the bacterial over the cytosolic ribosome can be explained by the increased repulsive interaction with the 1408G residue due to the increased steric hindrance at 6'-position. The 6'''-*N*-(2-hydroxyethyl) and 6'''-deamino-6'''-hydroxy

modifications of neomycin B cause relatively minor increase in ribosomal selectivities (**38** and **46**, Entries 3 and 4, Table 2). This is consistent with the fact that ring-IV of the 4,5-AGAs mainly contributes to binding through electrostatic interaction rather than through any specific interactions.¹⁶⁸⁻¹⁷⁰ The 4'-*O*-ethyl neomycin B show reduction in selectivity for the bacterial over the mitochondrial ribosome but an increase in selectivity over the mutant mitochondrial ribosome (**27**, Entry 6, Table 2). Whereas 4'-*O*-ethyl paromomycin **22** show a greater increase in selectivities for both the bacterial over mitochondrial and the mutant mitochondrial ribosomes (**22**, Entry 7, Table 2). In contrast, the combination of 6'-*N*-(2-hydroxyethyl) and 6'''-*N*-(2-hydroxyethyl) modification in a single molecule, **42**, does not cause an improvement in SelQ (Entry 8, Table 2), nor does the combination of 6'-*N*-(2-hydroxyethyl) and 4'-*O*-ethyl modifications in **53** (Entry 9, Table 2). The compounds **54** and **56** are the byproducts of synthetic and in both the compounds the 5''-hydroxy group is replaced by an ethanolamine linked via nitrogen. The key feature of these two compounds is the marked decrease in the selectivity for the bacterial over cytosolic ribosomes (Entries 10 and 11, Table 2). This is due to an increase in affinity for the cytosolic ribosome and earlier observed in 5''-deoxy-5''-amino derivative of 4,5-AGA ribostamycin.¹⁷¹⁻¹⁷³



Scheme 4. Synthesis of neomycin B derivatives **53**, **54**, and **56**.

Table 1. Antiribosomal activities (IC₅₀, µg/mL) and selectivities of modified neomycin B derivatives (selectivities are obtained by dividing the eukaryotic values by the bacterial values).

Compound	In vitro <i>M. smegmatis</i> (IC ₅₀ , µg/mL)			
	Bacterial	Mit13 (Selectivity)	A1555G (Selectivity)	Cyt14 (Selectivity)
Paromomycin 21	0.02	50.2 (2506)	5.39 (267)	9.4 (471)
22	0.08	99.3 (1240)	114.7 (1434)	102.9 (1286)
Neomycin B 9	0.01	1.62 (162)	0.22 (22)	17.12 (1712)
27	0.01	0.94 (94)	2.76 (276)	32.3 (3232)
37	0.01	13.11 (1311)	5.51 (551)	38.31 (3831)
38	0.01	9.72 (972)	0.74 (74)	21.51 (2151)
42	0.04	44.56 (1114)	11.96 (299)	76.33 (1908)
44	0.16	122 (776)	606 (3860)	295 (1879)
46	0.01	12.16 (1216)	1.13 (113)	54.71 (5471)
53	0.07	2.91 (41)	20.26 (289)	60.54 (865)
54	1.90	74.81 (39)	95.79 (50)	83.85 (44)
56	0.64	43.15 (67)	47.54 (74)	53.39 (83)

All the compounds were screened for their antibacterial activities against clinical isolates of methicillin-resistant Gram-positive bacterium *Staphylococcus aureus* (MRSA) and Gram-negative bacterium *Escherichia coli* and *Pseudomonas aeruginosa* obtained from the Diagnostic Division of the Institute of Medical Microbiology, University of Zurich. The MIC data against the clinical isolates of MRSA and *E. coli* is given in Table 3 and no significant activity was observed against clinical isolates of *P. aeruginosa*. Some compounds were also screened for their activity against the ESKAPE pathogens *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Acinetobacter baumannii* (Table 4). Most of the singly modified neomycin B derivatives showed antibacterial

activities comparable to that of the parent neomycin B against MRSA and *E. coli* (Table 3), and against the ESKAPE pathogens (Table 4). Doubly and triply modified neomycin B derivatives are relatively less active than the singly modified derivatives. It is important to note that compounds **27** and **53** carrying a 4'-*O*-ethyl modification retain good antibacterial activity against neomycin resistant strains of MRSA (Table 3).

Table 2. Change in antiribosomal selectivity (selectivity quotient = selectivity of derivative/selectivity of parent) of neomycin B derivatives with respect to parent neomycin B.

Entry	Compounds	Selectivity Quotient (SelQ)		
		Mit13	A1555G	Cyt14
1	Paromomycin 21	15.47	12.14	0.28
2	37	8.09	25.05	2.24
3	38	6.00	3.37	1.26
4	46	7.51	5.14	3.20
5	44	4.5	176	1.1
6	27	0.58	12.55	1.89
7	22	7.66	65.18	0.75
8	42	6.88	13.28	1.12
9	53	0.26	13.14	0.51
10	54	0.24	2.27	0.03
11	56	0.42	3.37	0.05

Table 3. Antibacterial activities of modified neomycin B derivatives (MIC, $\mu\text{g/mL}$).

Compound	MRSA				<i>E. coli</i>		
	AG038	AG039	AG042	AG044	AG001	AG055	AG003
Paromomycin 21	4	>256	>256	4-8	4	2-4	2-4
22	8-16	16	8-16	8-16	64	32-64	32
Neomycin 9	1-2	128	128	1	2	1-2	1
27	4	2	2	1-2	4	2-4	2-4
37	4	>128	>128	2	4	2-4	4
38	2	>128	>128	2	2-4	4	4
42	8	>128	>128	4	16-32	16	16
44	8	>64	>64	8-16	8	8	8
46	4	>128	>128	2	4	4	4
53	8-16	8	4-8	4-8	16	8-16	8-16
54	32-64	32-64	32	64	64-128	64-428	32-64
56	16	16-32	16	16	32	16-32	16-32

Table 4. Antibacterial activities (MIC, $\mu\text{g/mL}$) of modified neomycin B derivatives against ESKAPE pathogens.

Compound	<i>K. pneumoniae</i>			<i>E. cloacae</i>			<i>A. baumannii</i>		
	AG261	AG262	AG263	AG290	AG291	AG292	AG225	AG226	AG286
Paromomycin 21	1	1	1	2	1-2	2	4	2-4	1-2
22	4	8	8	8-16	8-16	8	16	16	32
Neomycin B 9	0.5	0.5	1	1	1	1	4	4	0.5-1

27	0.5	1	1	1	1	1	4	4	1-2
37	0.5-1	1	0.5	1	1	1	2	2	1
38	0.5	0.5	1	1	0.5	0.5	2	2	0.5
46	0.5	0.5	1	1	0.5-1	2	2	2	1
53	2-4	4	4	8	4	4	8-16	4-8	8

In order to determine the effectiveness of each modification in circumventing the modifications by common AMEs, all the synthesized Compounds were screened against wild type and recombinant *Escherichia coli* strains carrying defined resistance determinants³⁹ (Table 5). The 4'-O-ethylation as in the case of **27** and **53** is effective in evading the modifications caused by ANT-(4', 4'') class of AMEs and retains good antibacterial activity against resistant strains. This is consistent with the earlier reports in the paromomycin and other series.^{149,168,174,175} Most of the derivatives were not inactivated by the presence of either the AAC-(3) or AAC-(2') resistance determinants and this is consistent with the general lower susceptibility of the 4,5-AGAs than the 4,6-AGAs to AACs.¹¹¹ None of the modifications were helpful in circumventing the APH-(3', 5'') mechanism of resistance and compounds are completely inactive against these resistant strains. The ACC (6') resistance determinants cause about eight-fold decrease in antibacterial activity of neomycin B (Table 5) and this is in accordance with the literature reports that 6'-N-acetylation doesn't completely inactivate neomycin B, and also proved here by 6'-N-acetyl neomycin B (**44**).^{160,161,176} Compounds **27**, **38** and **46** display greater loss of activity in the presence of AAC-(6') class of AMEs. The introduction of 6'-N-(2-hydroxyethyl) modification (**37**, **42**, **53** and **56**) was able to effectively protect against AAC-(6') resistance mechanism.

Table 5. Antibacterial data of modified neomycin B derivatives against wild type and engineered strains of *E. coli* carrying specific resistance determinants (MIC, µg/ml).

Strain:	AG006	AG007	AG105	AG009	AG036	AG037
Resistance Mechanism:	Wild Type	AAC (3)	AAC (2')	AAC (6')	ANT (4', 4'')	APH (3', 5'')
Neomycin B 9	1	4	2	8	32	>256
27	0.5	2	2	32-64	0.5	>256
37	1	2	2	2	64	>256
38	1	2	2	16	32	>256
42	2	8	-	8-16	>64	>256
44	4-8	16	4	16	>64	>256
46	1	2	2-4	64	128	>256
53	2-4	8-16	8	16	4	>256
56	4	8	-	16-32	8	>256

2.1.4. Conclusion:

The 6'-*N*-(2-hydroxyethyl) modification of neomycin B doesn't cause any significant reduction in antibacterial activity and leads to enhanced selectivity for the bacterial ribosome over mitochondrial and mutant mitochondrial ribosomes. It also effectively suppresses the action of the AAC-(6') resistance mechanism. The 4'-*O*-ethyl modification of neomycin B also causes an increase in selectivity for prokaryotic over eukaryotic ribosomes and does not cause any reduction in antibacterial activity. This 4'-*O*-ethylation also blocks the activity of the ANT-(4', 4'') class of AMEs. Double and triple modifications of neomycin B are less advantageous as they generally show a greater loss in antibacterial activity and antiribosomal selectivity.

2.2. N2'-modifications to neomycin B.

2.2.1. Introduction:

After modifications of the 4', 6', and 6'''-positions in neomycin B, the 2'-position was targeted. The 2'-amino group present in most of the 4,5- and 4,6-series of AGAs, does not make any specific interaction with the bacterial A-site and mainly contributes to binding through electrostatic interactions.^{18,27,49,50,177} In neomycin B and other members of the 4,5-series of AGAs the protonated 2'-amino group has been considered to make an intramolecular hydrogen bond with the 5'''-hydroxy group that is hypothesized to enforce the correct conformation for the optimal interaction of rings-I and II with the bacterial A-site (Figure 21).^{135,177} Conversely, the 2'-amino group makes neomycin B and other members of the 4,5- and 4,6-series of AGAs susceptible to inactivation by the AAC-(2') class of AMEs.^{116,166,178-182} There are only limited examples in the literature of 2'-modifications to AGAs and their influence on antibacterial activity. 2'-N-Alkylated derivatives of sisomicin retain their antibacterial activities and 2'-N-ethyl netilimicin has a comparable antibacterial activity to netilimicin itself, neither are susceptible to modifications by the AAC-(2') class of AMEs.¹⁸³⁻¹⁸⁸ Similarly, 2'-N-ethyl paromomycin has also been shown to possess comparable activity to paromomycin.¹⁸⁹ Kanamycin A, with a 2'-hydroxy substituent has a similar antibacterial profile as kanamycin B, with its 2'-amino substituent, and is not affected by most of the AAC-(2') class of AMEs.^{27,117,166,168,190} However, it has been reported that AAC-(2')-Ic from *M. tuberculosis* can catalyze the acetyl-CoA dependent acetylation of kanamycin A and amikacin, both of which feature a 2'-hydroxyl substituent, suggesting that this AME can catalyze *O*-acetylation.¹⁸² It has also been reported that the 2'-deamino-2'-hydroxy modification of neomycin B is not detrimental to antibacterial activity.¹⁶² In contrast to the above limited but generally encouraging literature precedent on the modification of neomycin B at the 2'-position

with regard to activity, nothing is known of the effect of this type of modification on ribosomal selectivity and hence toxicity. Modification of the neomycin B 2'-position was therefore considered a reasonable avenue of exploration in the search of novel AGAs with an improved pharmacological profile. Accordingly, several 2'-*N*-alkylated, 2'-deamino, and 2'-amido derivatives were synthesized, and their antiribosomal and antibacterial activities were screened.

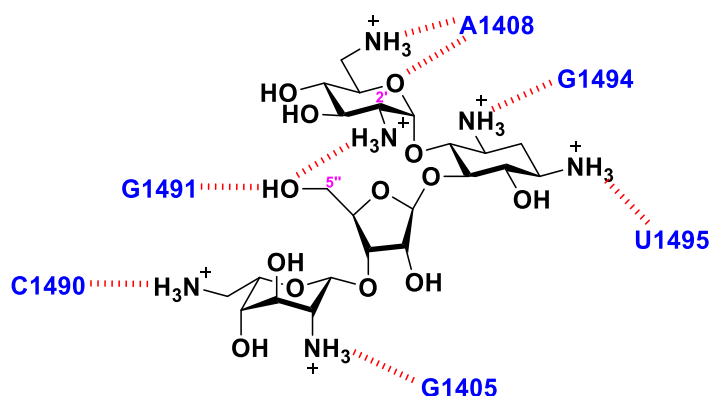


Figure 21. Schematic representation of intramolecular 2'-5''-hydrogen bond in neomycin B and its interactions with bacterial 16S r-RNA.

2.2.2. Synthesis:

The 2'-*N*-methyl and 2'-*N*-ethyl neomycin B derivatives **59** and **61** were readily prepared from neomycin B. Following a literature procedure,^{162,189} treatment of neomycin B free base with acetic anhydride in the presence of equimolar 1N HCl in aqueous methanol gave penta-*N*-acetyl neomycin B derivative **57** as the major product in 39% yield. The observed regioselectivity can be attributed to the difference in the basicity of the amino groups and the steric environments. Double one pot reductive amination of **57**, first with benzaldehyde and then with formaldehyde using sodium cyanoborohydride as reducing agent¹⁵⁹ gave a crude preparation of the corresponding 2'-*N*-benzyl-*N*-methyl derivative, which was per-acetylated using acetic anhydride in pyridine to give

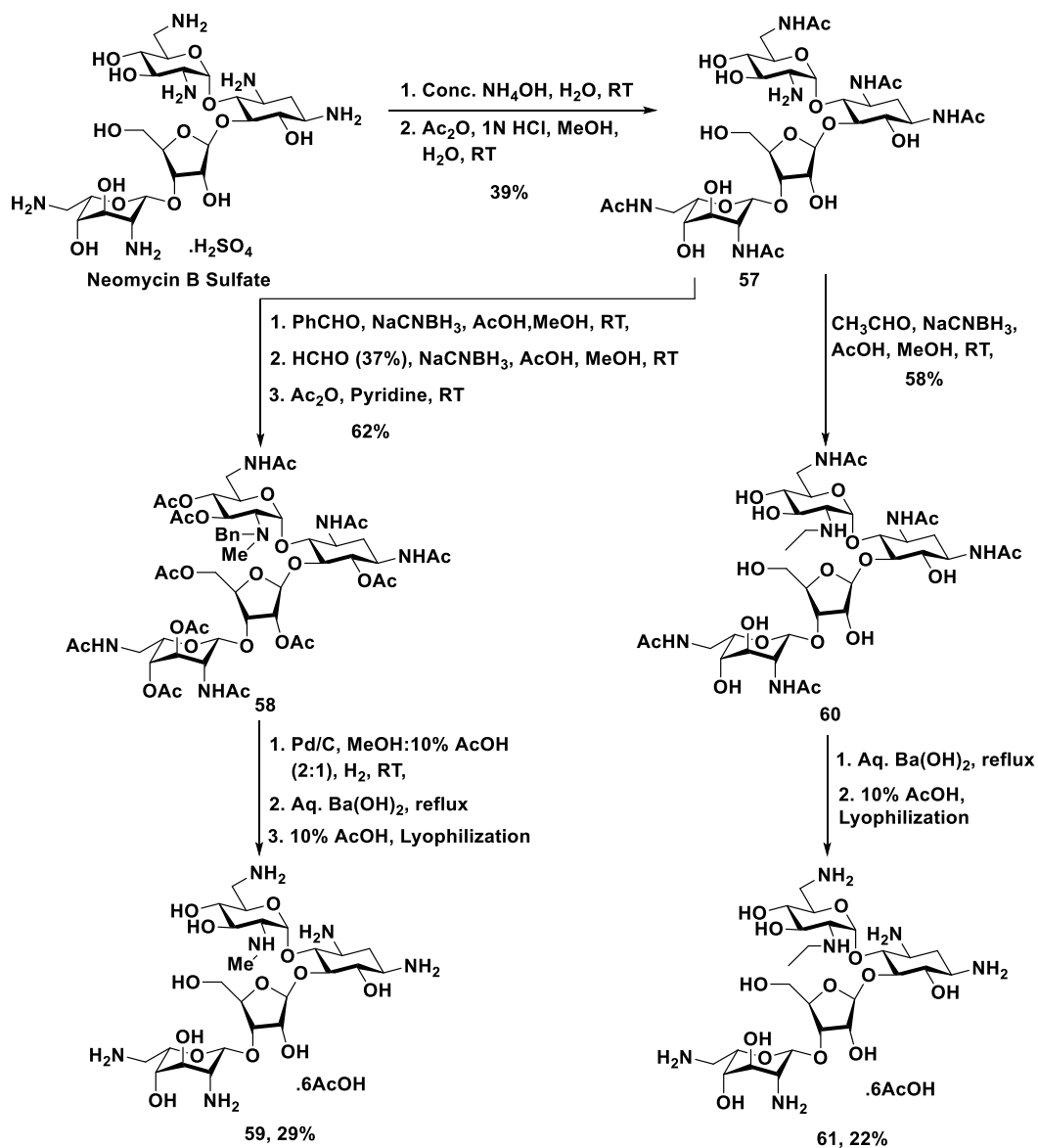
the fully protected intermediate **58** with an overall 62% yield for the three steps. Finally, application of a two-step deprotection procedure, involving hydrogenolysis to remove benzyl group and saponification of the acetyl groups in hot aqueous barium hydroxide, and then purification on Sephadex and lyophilization from aqueous acetic acid gave the 2'-*N*-methyl neomycin B **59** in the form of its peracetate salt with 29% yield (Scheme 5). Similarly, reductive amination of **57** with acetaldehyde gave the 2'-*N*-ethylated intermediate **60** in 58% yield, which upon saponification in hot aqueous barium hydroxide, and then purification on Sephadex and lyophilization from aqueous acetic acid gave the 2'-*N*-ethyl neomycin B **61** in the form of its peracetate salt in 22% yield (Scheme 5). The precise location of the methyl and ethyl group was confirmed by heteronuclear multiple bond coherence (HMBC) correlations between the methyl group and the 2'-position in the case of 2'-*N*-methyl neomycin B **59**, and between the ethyl methylene group and the 2'-position in the case of 2'-*N*-ethyl neomycin B **61**.

For the synthesis of 2'-deamino neomycin B **64**, the neomycin B pentaacetamide **57** was treated with acetic formic anhydride¹⁹¹ and pyridine in *N,N*-dimethylformamide to give the corresponding 2'-formamido intermediate and this crude intermediate was per-acetylated using acetic anhydride in pyridine to give the fully protected 2'-formamido derivative **62** with an overall 65% yield for the two steps. Dehydration of the 2'-formamido derivative **62** with phosphoryl chloride and triethyl amine in dichloromethane gave the corresponding isonitrile intermediate, which was subjected to radical deamination conditions using tris(trimethylsilyl) silane (TTMS) and azobisisobutyronitrile (AIBN) in a hot mixture of toluene and acetonitrile to give the fully acetylated 2'-deamino intermediate **63** with an overall 44% yield for the two steps (Scheme 6). The mechanism for the replacement of the isonitrile moiety with a hydrogen atom is shown in Scheme 7. AIBN serves as the radical initiator, and upon heating generates two isobutyronitrile

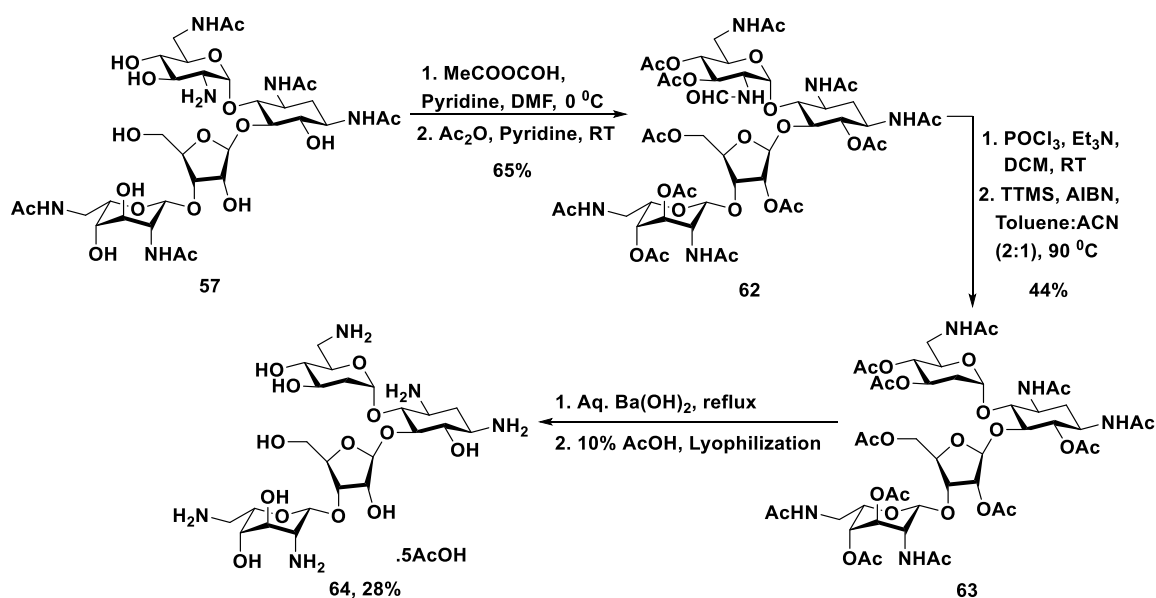
radicals with the liberation of a nitrogen molecule. The isobutyronitrile radical abstracts a hydrogen atom from TTMS to generate the tris(trimethylsilyl)silyl radical, which adds to the isonitrile to give an imidoyl radical intermediate. This imidoyl radical undergoes fragmentation to generate the alkyl radical and this alkyl radical abstracts a hydrogen atom from TTMS to give the corresponding alkane along with another tris(trimethylsilyl)silyl radical (Scheme 7).¹⁹²⁻¹⁹⁴ Saponification of the acetyl groups in hot aqueous barium hydroxide, and then purification on Sephadex and lyophilization from aqueous acetic acid gave the 2'-deamino neomycin B **64** as the peracetate salt in 28% yield (Scheme 6).

For the synthesis of 2-deamino-2'-hydroxy neomycin B **70**,¹⁶² a glycosylation strategy was adopted. The glycosyl acceptor **67** was synthesized from paromomycin as described in the literature.^{162,189} Treatment of paromomycin free base with acetic anhydride in the presence of equimolar 1N HCl in aqueous methanol gave the tetra-*N*-acetyl paromomycin derivative **65** as a major product with 40% yield. Protection of the 2'-amino group as the benzyl carbamate using benzyl chloroformate and then per-acetylation using acetic anhydride in pyridine gave the fully protected intermediate **66** with an overall 57% yield for the two steps. Finally, removal of the benzyl carbamate by hydrogenolysis gave the 2'-amino intermediate, which upon diazotization with sodium nitrite in aqueous acetic acid smoothly gave the glycosyl acceptor **67** with an overall 52% yield for the two steps (Scheme 8). The selective cleavage of ring I in this manner confirms the regioselectivity of the acetamide forming process when conducted in the presence of dilute HCl. The formation of the glycosyl acceptor from the 2'-amine is proposed to proceed via the formation of an unstable diazonium salt, which rearranges to a fused bicyclo oxonium ion with expulsion of nitrogen. The three membered ring is then opened with the aid of a lone pair on the glycosidic oxygen affording an oxocarbenium ion, whose hydrolysis results in the glycosyl

acceptor **67** (Scheme 9).^{163,195,196}

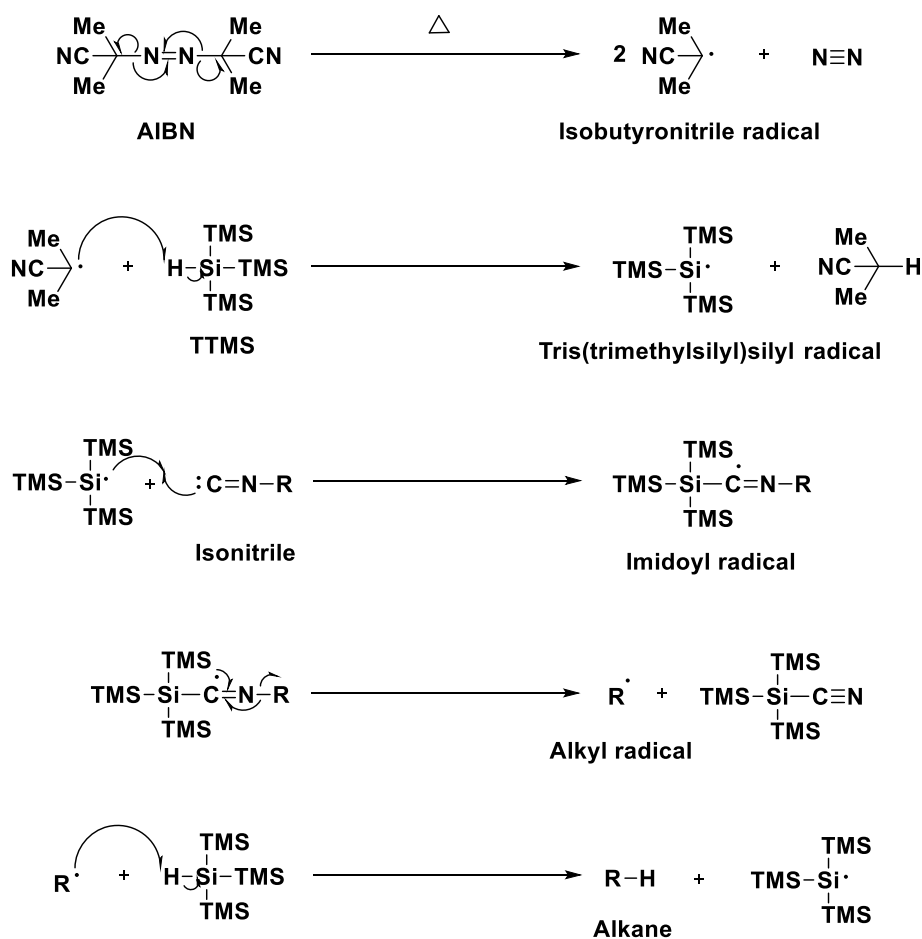


Scheme 5. Synthesis of 2'-*N*-alkyl neomycin B derivatives **59** and **61**.



Scheme 6. Synthesis of 2'-deamino neomycin B **64**.

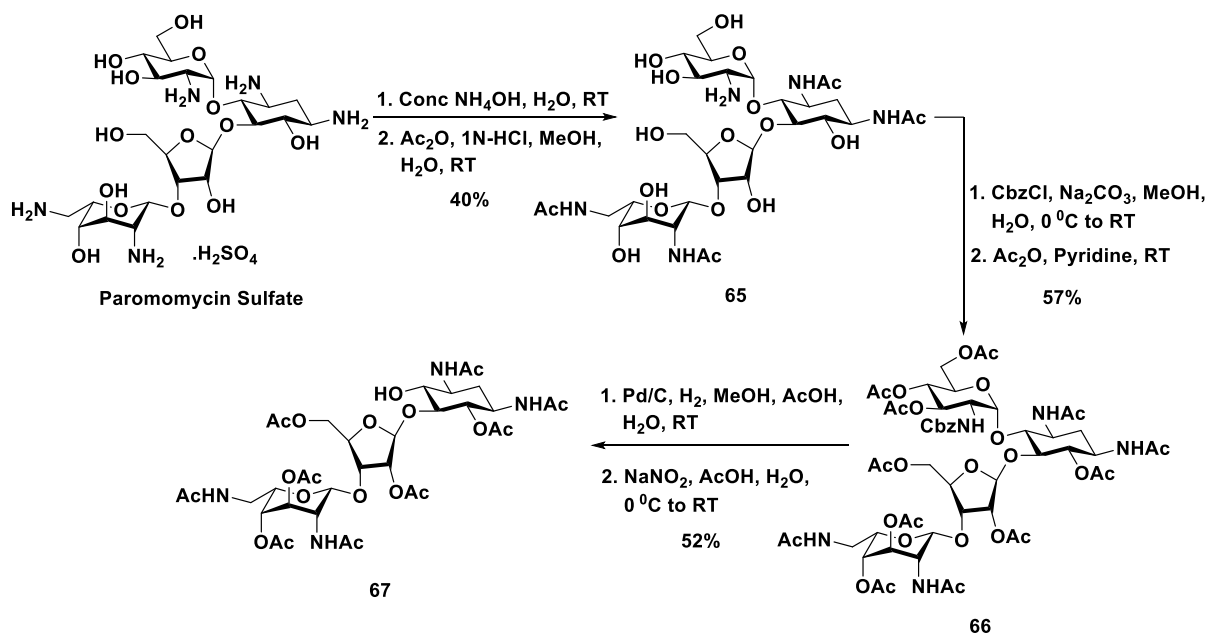
The glycosyl acceptor **67** and thioglycoside **68**¹⁵³ were coupled using *N,N*-dimethylformamide modulated highly α -selective glycosylation conditions.¹⁹⁷ The thioglycoside **68** was activated by *N*-iodosuccinimide and trimethylsilyl triflate in the presence of *N,N*-dimethylformamide to give an intermediate glycosyl imidate, which upon nucleophilic attack by glycosyl acceptor **67** gave the glycosylated product **69** in 29% yield (Scheme 10). It is proposed that activation of the thioglycoside generates an oxacarbenium ion pair that is trapped by *N,N*-dimethylformamide to give an equilibrium mixture of α - and β -glycosyl imidates. Subsequent nucleophilic attack of the glycosyl acceptor on the more reactive β -glycosyl imidate gives the desired α -glycosylation product as the major product (Scheme 11).¹⁹⁷ Finally, application of a two-step deprotection procedure, involving hydrogenolysis of the benzyl ethers and azides, followed by saponification in hot aqueous sodium hydroxide, and then purification on Sephadex and lyophilization from aqueous acetic acid gave the 2'-deamino-2'-hydroxy neomycin B **70** as the peracetate salt in 34% yield (Scheme 10).



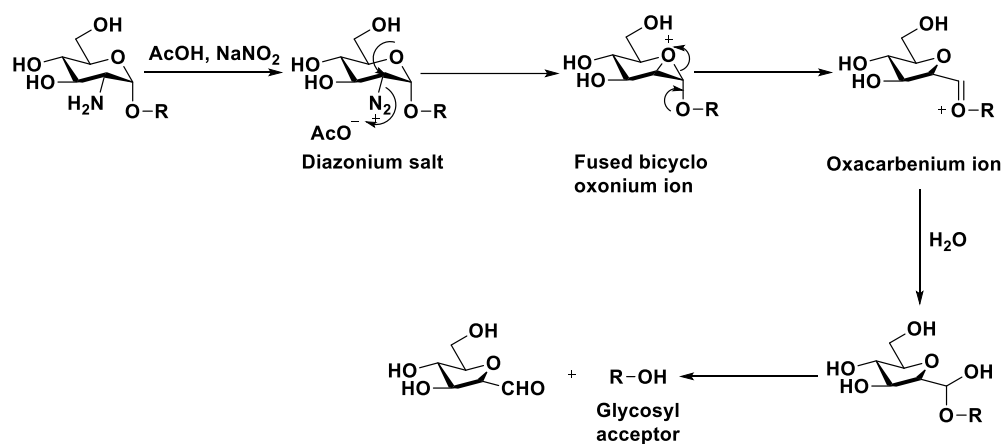
Scheme 7. Proposed radical mechanism for replacement of isonitrile with a hydrogen atom (R-NC represents a fully acetylated neomycin B derivative with a isonitrile group at 2'-position).

The synthesis of 2'-*N*-(2-aminoacetyl)-neomycin B **75** started with the selective acetylation of neomycin B using the conditions described before to give penta-*N*-acetyl neomycin B derivative **57**. The crude preparation of **57** was treated with imidazolesulfonyl azide^{157,158} in the presence of potassium carbonate and catalytic copper sulfate to give the corresponding 2'-azido derivative. Then further reaction of the amidic nitrogen atoms with di-*tert*-butyl dicarbonate and 4-(dimethylamino) pyridine in hot tetrahydrofuran, followed by protection of the hydroxy groups with acetic anhydride in pyridine gave the fully protected neomycin B derivative **71** with an overall 13% yield for the four steps (Scheme 12). Cleavage of all the acetyl groups with sodium methoxide

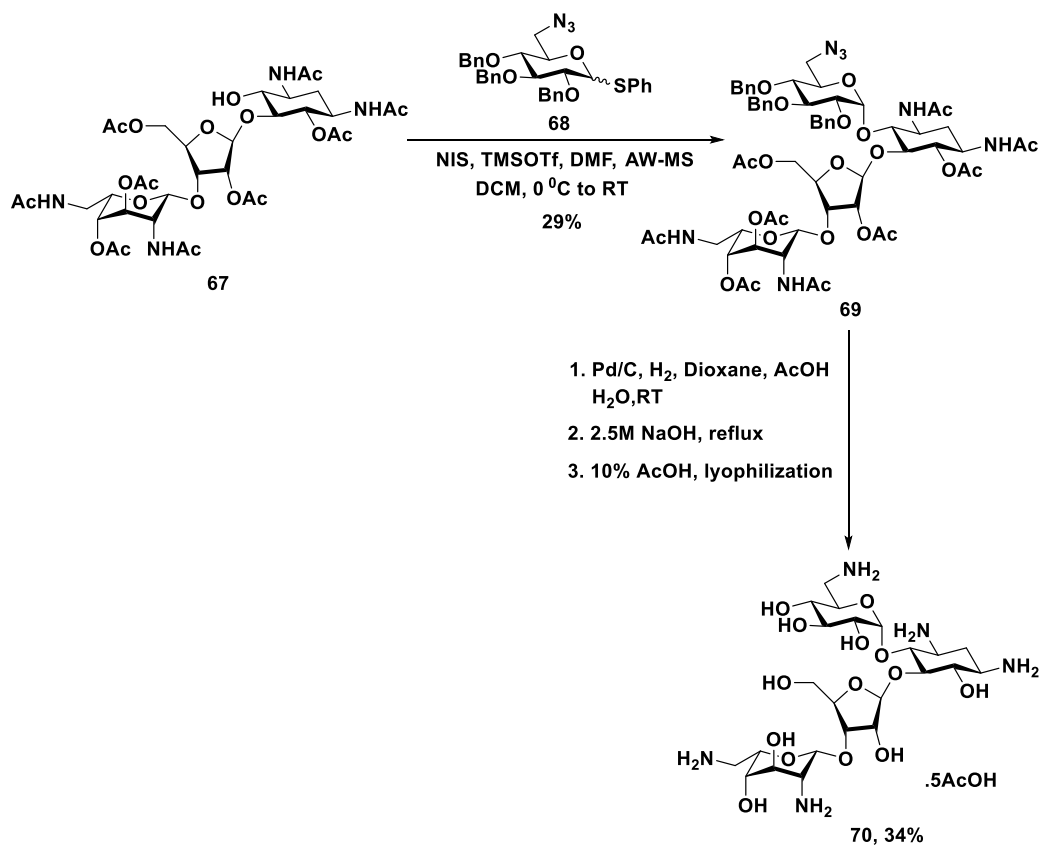
in methanol, followed by Staudinger reaction with trimethylphosphine¹⁵⁵ to reduce 2'-azide gave the penta-*N*-tert-butoxycarbonyl-neomycin B **72** in 53% yield over two steps. Subsequent treatment of **72** with 2-azidoacetic acid **73**, *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride and 1-hydroxybenzotriazole gave the intermediate **74** in 68% yield. Finally, application of two step deprotection protocol, involving azide reduction by hydrogenolysis, followed by cleavage of the *tert*-butyl carbamates with trifluoroacetic acid,¹⁶⁴ and then purification on Sephadex and lyophilization from aqueous acetic acid gave the 2'-*N*-(2-aminoacetyl)-neomycin B **75** in the form of its peracetate salt in 48% yield (Scheme 12). The precise location of the 2-aminoacetyl group was confirmed by heteronuclear multiple bond coherence (HMBC) correlation between the carbonyl carbon of the 2-aminoacetyl group and the hydrogen atom at the 2'-position. The corresponding 2'-*N*-formyl neomycin B **76** and 2'-*N*-acetyl neomycin B **77** (Figure 22) derivatives were also synthesized analogously from intermediate **72** by Dr. Vikram Sarpe in the Crich laboratory for comparison purposes.



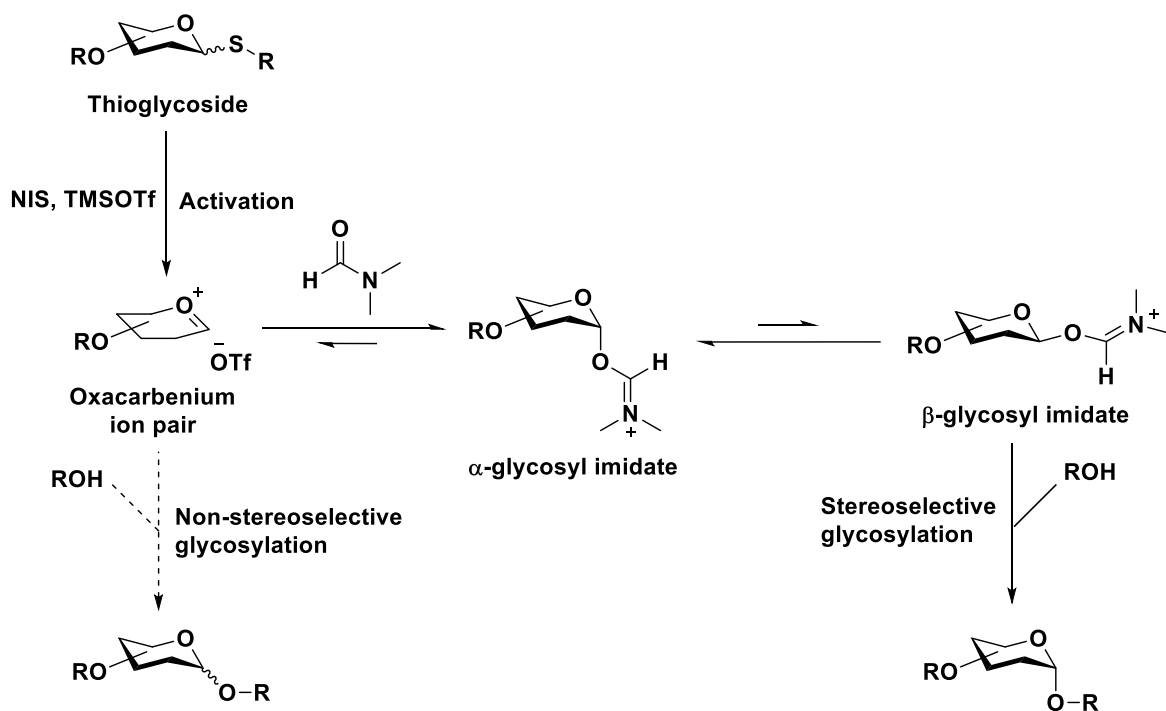
Scheme 8. Synthesis of glycosyl acceptor **67**.



Scheme 9. Proposed mechanism for the formation of glycosyl acceptor **67** from the 2'-amino intermediate (R = ring II, III and IV of paromomycin derivative **66**).



Scheme 10. Synthesis of 2'-deamino-2'-hydroxy neomycin B **70**.



Scheme 11. Proposed mechanism for DMF mediated α -selective glycosylation.

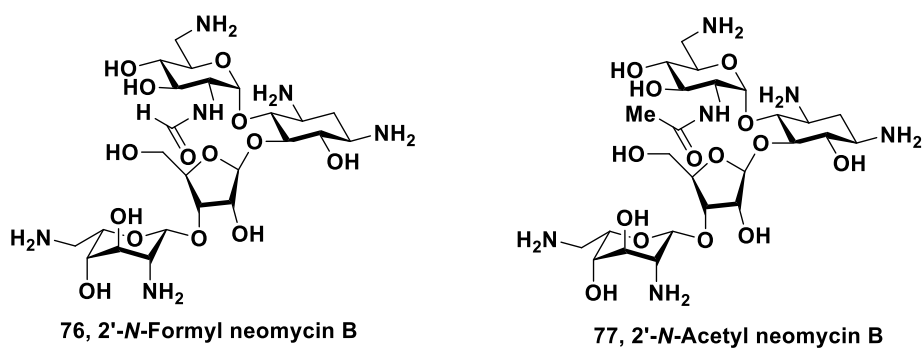
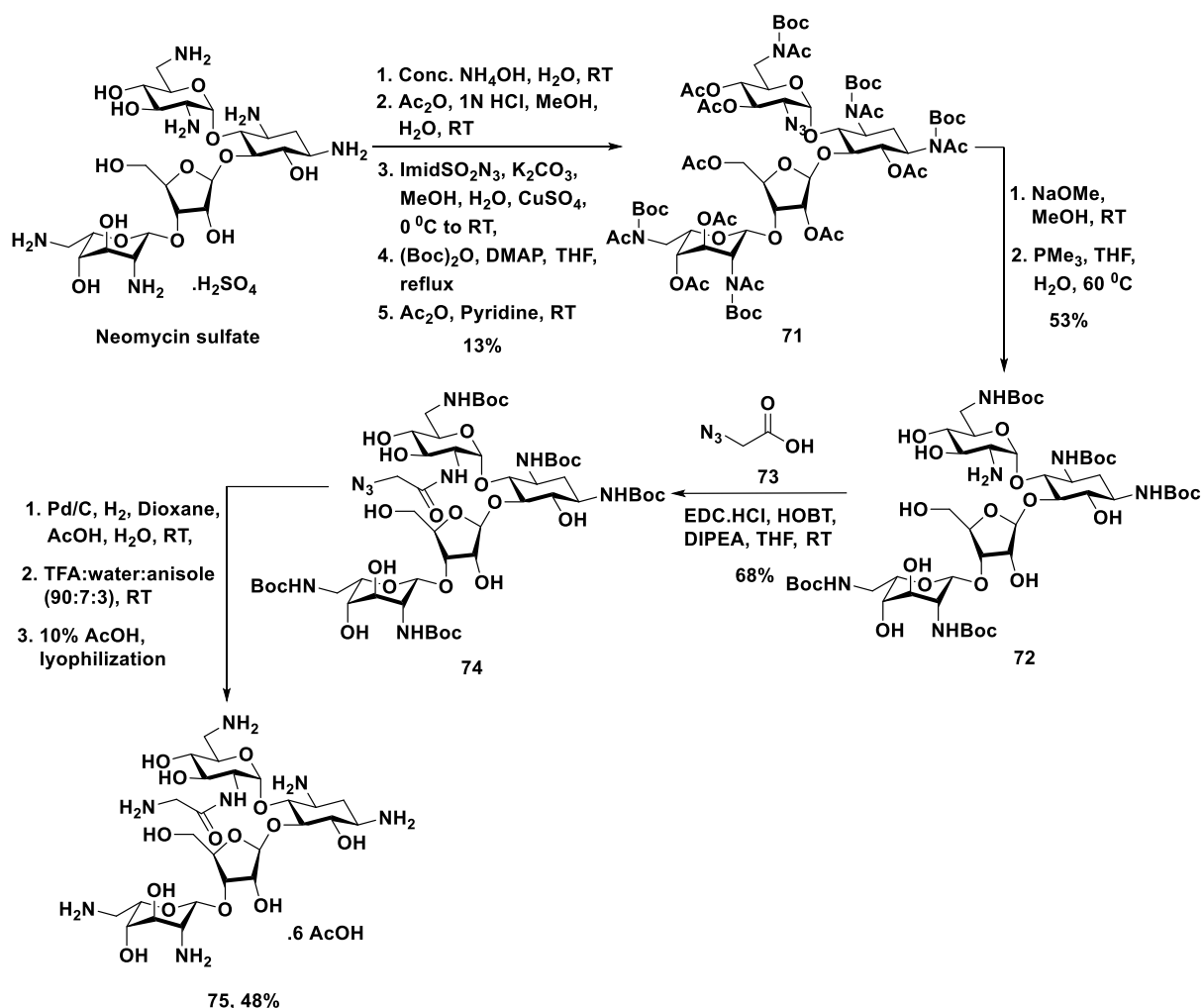


Figure 22. Structures of 2'-N-formyl neomycin B 76 and 2'-N-acetyl neomycin B 77.



Scheme 12. Synthesis of 2'-*N*-(2-aminoacetyl)-neomycin B **75**.

2.2.3. Biological evaluations:

The newly synthesized 2'-modified neomycin B derivatives **59**, **61**, **64**, **70**, and **75** were screened for their antiribosomal activities in cell free translational assays employing wild type bacterial ribosomes and recombinant hybrid bacterial ribosomes (Table 6). Their antiribosomal activities were also compared with those of the parent neomycin B **9**, of 2'-*N*-formyl neomycin B **76**, and of 2'-*N*-acetyl neomycin B **77** (Table 6). The activity of neomycin B against the bacterial ribosome is not affected by 2'-*N*-alkylation (**59** and **61**, Table 6), which is consistent with the earlier reports on the antibacterial activity of 2'-*N*-alkylated derivatives of sisomicin series¹⁸³⁻¹⁸⁸

and 2'-*N*-ethyl paromomycin.¹⁸⁹ In these compounds the secondary amines at the 2'-position can still be protonated and contribute to binding to the bacterial A-site. These results also demonstrate that the bacterial ribosomal binding A-site can accommodate at least a two carbon substituent at this position. The 2'-deamino neomycin B **64** also shows no reduction in activity against the bacterial ribosome, which invalidates the literature hypothesis on the importance of the 2'-5''-hydrogen bond in enforcing the correct conformation for binding. Continuing the trend, the 2'-deamino-2'-hydroxy derivative **70** shows comparable activity against the bacterial ribosome to the parent neomycin B **9**, consistent with the earlier report on antibacterial activity of **70**.¹⁶² In contrast, all the 2'-amido derivatives **75**, **76**, and **77** showed a marked decrease in their activity against the bacterial ribosomes. Presumably, this is due to the fact that the amidic nitrogen is not basic, is not protonated at physiological pH, and cannot contribute to binding through electrostatic interaction. The 2'-*N*-formyl derivative **76** shows slightly better activity as compared to 2'-*N*-(2-aminoacetyl) derivative **75** and 40-fold higher activity than the 2'-*N*-acetyl derivative **77**. This is presumably due to steric reasons as the formyl group is sterically less cumbersome than the acetyl and 2-aminoacetyl groups. The 2'-*N*-(2-aminoacetyl) **75** and 2'-*N*-acetyl **77** modifications have similar steric bulk but the 2'-*N*-(2-aminoacetyl) derivative **75** is 30-fold more active than the 2'-*N*-acetyl compound **77**. This is due to the presence of the additional basic amine in 2'-*N*-(2-aminoacetyl) group, which can be protonated under physiological conditions and so contribute electrostatically to binding.

Table 6. Antiribosomal activities (IC_{50} , μM) and selectivities of 2'-modified neomycin B derivatives (selectivities are obtained by dividing the eukaryotic values by the bacterial values).

Compound	In vitro <i>M. smegmatis</i> (IC_{50} , μM)			
	Bacterial	Mit13 (Selectivity)	A1555G (Selectivity)	Cyt14 (Selectivity)
Neo B 9	0.04	4.3 (108)	0.4 (10)	35 (875)
59	0.01	4.7 (470)	1.1 (110)	37 (3700)
61	0.01	11 (1100)	1.6 (160)	43 (4300)
64	0.03	22 (733)	0.9 (30)	85 (2833)
70	0.03	36 (1200)	2.7 (90)	108 (3600)
75	0.16	11 (69)	1.2 (75)	25 (156)
76	0.12	54 (450)	13 (108)	127 (1058)
77	5.3	93 (17)	28 (5)	147 (28)

To facilitate comparison of the influence of each modification on ribosomal selectivity, the raw selectivity data presented in Table 6 have been converted into selectivity quotients (SelQ) with respect to the parent neomycin as presented in Table 7. It is evident that the 2'-*N*-alkyl modification of neomycin B increases selectivity for the bacterial over mutant mitochondrial ribosome to a greater extent than over the wild type mitochondrial ribosome (**59** and **61**, entries 1 and 2, Table 7). Probably, the looser wild type mitochondrial A-site better accommodates the 2'-*N*-alkyl groups than the tighter and more rigid mutant mitochondrial A-site. The 2'-deamino and 2'-deamino-2'-hydroxy derivatives show only a modest increase in selectivity pattern (**64** and **70**, entries 3 and 4, Table 7). Compounds **64** and **70** also show a modest increase in selectivity for the bacterial over the cytosolic ribosome as is the case for the 2'-*N*-alkylated derivatives **59** and **61**. The 2'-*N*-(2-aminoacetyl) derivative also showed greater increase in selectivity for the bacterial over mutant mitochondrial ribosome than over the wild type mitochondrial ribosome (**75**, Entry 5, Table 7).

This can also be explained in terms of the looser wild type mitochondrial A-site better accommodating the 2'-*N*-(2-aminoacetyl) group as compared to the rigid mutant mitochondrial A-site. The 2'-*N*-formyl and 2'-*N*-acetyl derivatives (**76** and **77**, entries 6 and 7, Table 7) showed a similar pattern of ribosomal selectivity as the 2'-*N*-(2-aminoacetyl) derivative **75**. It is interesting to note that the 2'-*N*-(2-aminoacetyl) **75** and 2'-*N*-acetyl **77** showed a significant decrease in selectivity for the bacterial over cytosolic ribosome due to a comparatively smaller change in affinity for the cytosolic ribosomes. The 2'-*N*-formyl derivative **76** showed a minor increase in selectivity for the bacterial over cytosolic ribosome.

All compounds were screened for their antibacterial activities against methicillin-resistant clinical isolates of the Gram-positive bacterium *Staphylococcus aureus* (MRSA) and against clinical isolates of the Gram-negative bacterium *Escherichia coli* and *Pseudomonas aeruginosa*, obtained from the Diagnostic Division of the Institute of Medical Microbiology, University of Zurich (Table 8). The 2'-*N*-alkylated **59** and **61**, 2-deamino **64** and 2-deamino-2'-hydroxy **70** derivatives showed similar antibacterial profiles as the parent neomycin B against MRSA and *E. coli*. On the other hand, the 2'-*N*-formyl derivative **76** showed better antibacterial activity in some of the MRSA and *E. coli* strains as compared to the 2'-*N*-(2-aminoacetyl) and 2'-*N*-acetyl derivatives **75** and **77**, consistent with the pattern of antiribosomal activities. No significant activity was observed against the clinical isolates of the Gram-negative bacterium *Pseudomonas aeruginosa*.

Table 7. Change in antiribosomal selectivity (selectivity quotient = selectivity of derivative/selectivity of parent) of 2'-modified derivatives with respect to parent neomycin B.

Entry	Compounds	Selectivity Quotient (SelQ)		
		Mit13	A1555G	Cyt14
1	59	4.35	11	4.23
2	61	10.18	16	4.9
3	64	6.79	3	3.24
4	70	11.11	9	4.11
5	75	0.64	7.5	0.18
6	76	4.17	10.8	1.21
7	77	0.16	0.5	0.03

Table 8. Antibacterial activities of 2'-modified neomycin B derivatives (MIC, µg/mL).

Compound	MRSA				<i>E. coli</i>			<i>P. aeruginosa</i>		
	AG038	AG039	AG042	AG044	AG001	AG055	AG003	AG031	AG032	AG033
Neo B 9	0.25	128	128	1	1	1-2	1	8	8	64
59	0.5	>32	>32	0.54	1	1	1	64	64	>64
61	0.5	>32	>32	1	1-2	1	1	>64	>64	>64
64	1	32	32-64	2	2	2	1-2	32	64	>64
70	2	>128	>128	2	2-4	2-4	2-4	128	128	>128
75	4	>32	>32	-	16	16	32	>32	>32	>32
76	2-4	>32	>32	-	4-8	2	4-8	>32	>32	>32
77	>32	>32	>32	-	>32	>32	>32	>32	>32	>32

All Compounds were also screened against wild type and recombinant *Escherichia coli* strains carrying defined resistance determinants³⁹ in order to determine their susceptibility to common AMEs (Table 9). The 2'-*N*-methyl and 2'-*N*-ethyl derivatives **59** and **61** completely evade

modification by the AAC-(2') resistance mechanism and show strong antibacterial activity against the resistant strain, which is consistent with the earlier reports in the sisomicin series that 2'-*N*-alkylated derivatives retain their activity against AAC-(2') class of AMEs.¹⁸³⁻¹⁸⁸ The 2'-deamino **64** and 2'-deamino-2'-hydroxy **70** derivatives, both lacking the 2'-amino substituent retain their antibacterial activity and are not susceptible to modification by AAC-(2') class of AMEs. The 2'-*N*-(2-aminoacetyl) derivative **75** does not show good antibacterial activity in general for either wild type or resistance strains, similar to 2'-*N*-acetyl neomycin B **77**, which is a completely inactive compound that validates the concept of inactivation by the AAC-(2') class of AMEs.^{116,166,178-182} The 2'-*N*-formyl derivative **76** retains antibacterial activity against resistant strains bearing AAC-(2'). None of the 2'-modifications were helpful in providing protection from modification by AAC-(6'), ANT-(4', 4''), and APH-(3', 5'') classes of AMEs. None of the 2'-modifications of neomycin B are not affected by the 16s-rRNA methyl transferase as demonstrated by the *E. coli* strain carrying ArmA mechanism for which they show similar antibacterial activity as for wild type strain.

Table 9. Antibacterial data of 2'-modified neomycin B derivatives against wild type and engineered strains of *E. coli* carrying specific resistance determinants (MIC, µg/ml).

Strain:	AG006	AG106	pH434	AG009	AG036	AG037	pGB2
Resistance Mechanism:	Wild Type	AAC (2')-Ia	AAC (2')-Ib	AAC (6')	ANT (4', 4'')	APH (3', 5'')	ArmA
Neo B 9	0.5-1	8	>64	4	4-8	>64	0.25-0.5
59	0.5	0.5	4	16-32	16	>64	1
61	0.5-1	1	4	32	16	>64	1
64	1	1	8	>64	≥64	>64	1
70	0.5-1	1	4	>64	64-128	>64	1
75	8-16	16-32	-	>256	32	>256	16

76	2	2	8-16	>64	64	>64	8
77	128	128	-	>512	256	>512	128-156

2.2.4. Conclusion:

The 2'-*N*-alkylation, 2'-deamino, and 2'-deamino-2'-hydroxy modifications of neomycin B do not cause any significant reduction in antibacterial activity and lead to moderately enhanced selectivity for the bacterial ribosome over mitochondrial and mutant mitochondrial ribosomes. These modifications also effectively suppress the action of the AAC (2') resistance determinants. The 2'-amido modifications are less advantageous as they generally result in both reduced antibacterial activities and selectivities.

CHAPTER 3. MODIFICATIONS TO PAROMOMYCIN.

3.1. N2'-modifications to paromomycin.

3.1.1. Introduction:

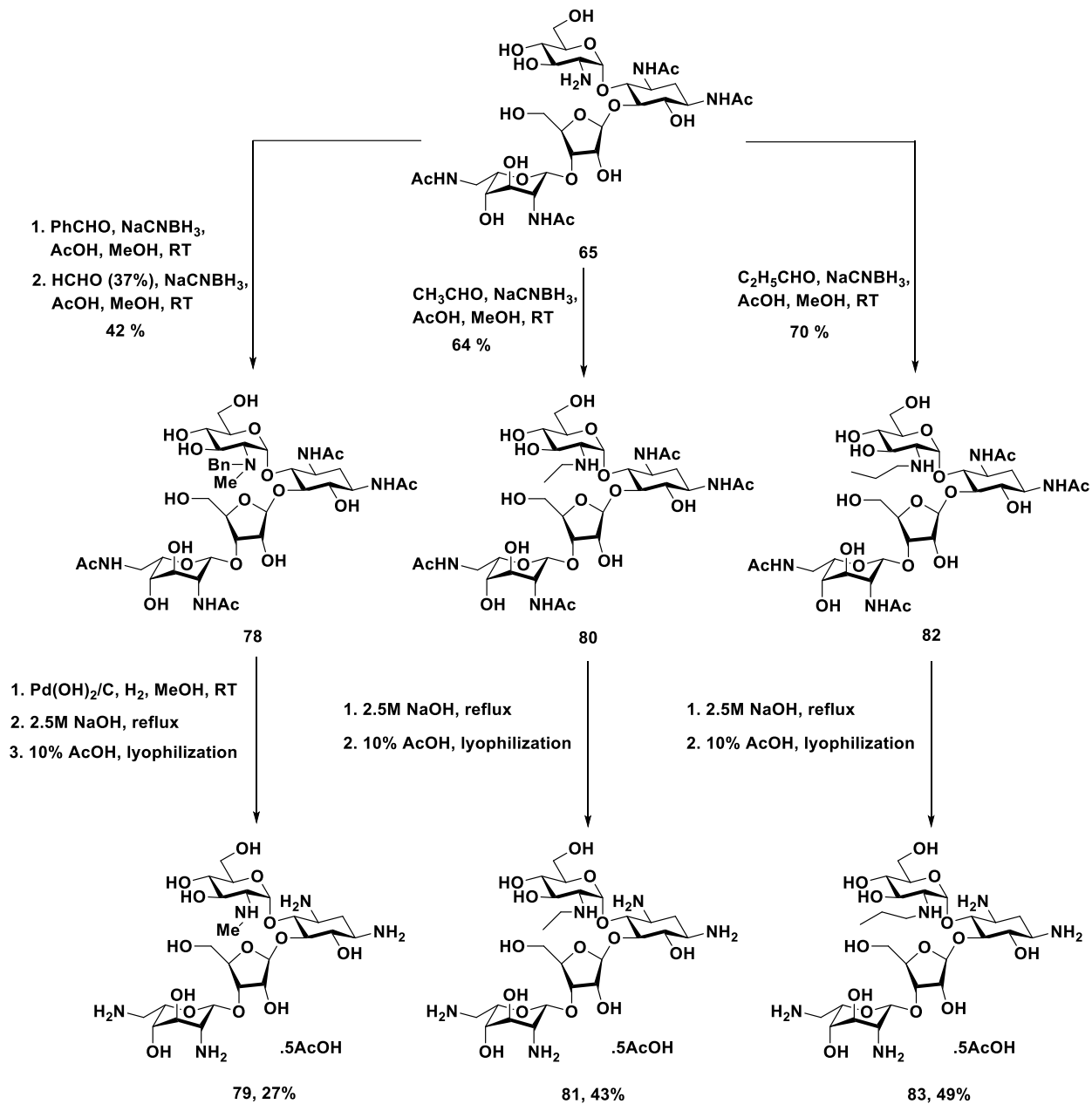
Paromomycin belongs to the 4,5-series AGAs and is a broad-spectrum antibiotic. It is also active against some protozoa and cestodes.¹⁹⁸ It was first discovered from *Streptomyces krestomuceticus* in 1950's and came into medical use in 1960's.^{198,199} It has been used to treat intestinal infections such as cryptosporidiosis, amoebiasis, and other diseases like leishmaniasis. It is also included on the WHO's list of essential medicines. The medical use of paromomycin was reduced due to the continued emergence of resistant bacteria and the inherent adverse effects, mainly ototoxicity. The only difference between neomycin B and paromomycin is the 6'-substituent, an amino group in neomycin B and a hydroxy group in paromomycin. This change of a 6'-amino to a 6'-hydroxy groups brings some advantageous effects to paromomycin over neomycin B as it does not cause a significant reduction in antibacterial activity but renders paromomycin significantly less ototoxic.^{55,56,70,74,75,138} Paromomycin is also not affected by the AAC-(6') resistance mechanism as it lacks the 6'-amino substituent.^{110,139-148} However, the disadvantage is that paromomycin shows stronger affinity towards the cytosolic A-site than neomycin B. This is because the 6'-hydroxyl group of paromomycin can accept the hydrogen bonds from the 1408G residue in cytosolic ribosome in contrast to the repulsive interaction between protonated 6'-amino group of neomycin B and G1408.^{166,167} The same 6'-OH-G1408 interaction is responsible for the paromomycin's antiprotozoal activity as the decoding A-site of protozoa are related in structure to the human cytosolic A-site.^{173,200} In recent years, several reports have been published for development of improved paromomycin derivatives. Most recently, certain 4'-O-alkyl, 4',6'-O-alkylidene, and 4'-O-glycosyl derivatives have been shown to have

comparable antibacterial activities to the parent and to cause little to no ototoxicity.^{167,174,201} Extrapolating from the studies in the neomycin series, and the earlier report on the antibacterial activity of 2'-*N*-ethyl paromomycin,¹⁸⁹ the 2'-position in paromomycin was targeted. Thus, several 2'-*N*-alkylated derivatives and 2'-deamino-2'-hydroxy variant were synthesized, and screened for their antiribosomal and antibacterial activities.

3.1.2. Synthesis:

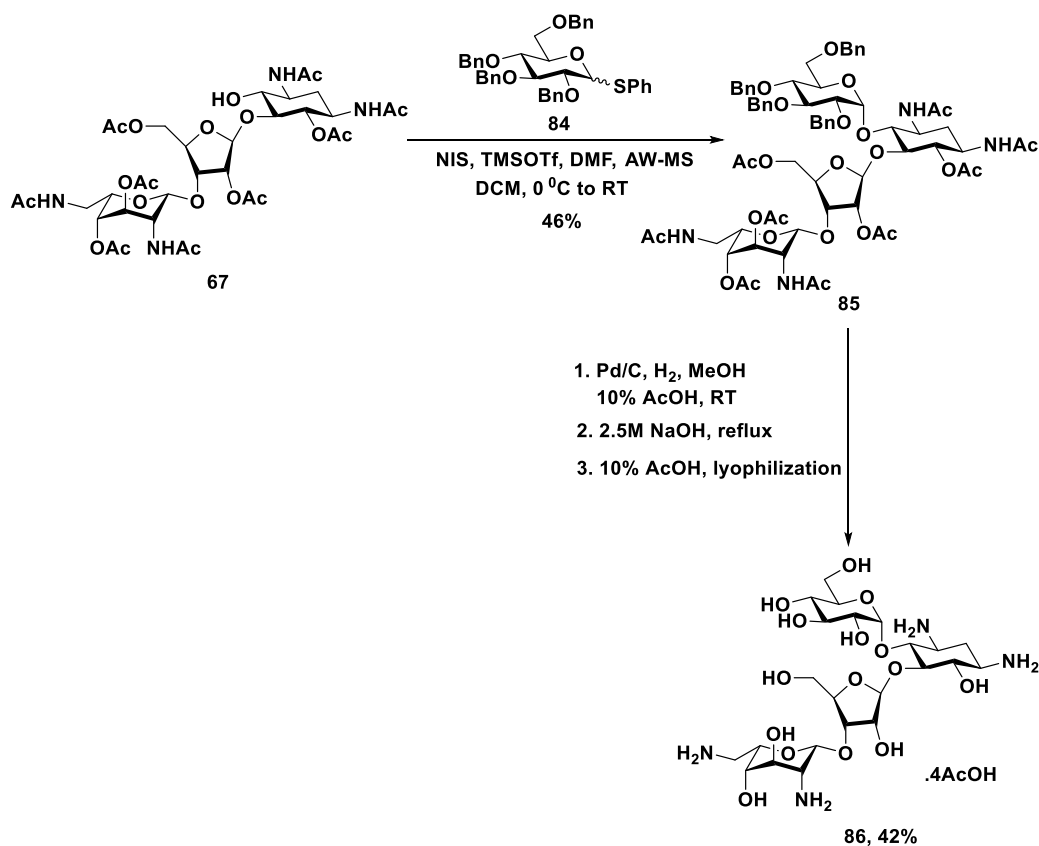
The 2'-*N*-methyl, 2'-*N*-ethyl, and 2'-*N*-propyl paromomycin derivatives **79**, **81**, and **83** were readily prepared from tetra-*N*-acetyl paromomycin intermediate **65**. Following the same one pot double reductive amination conditions applied for the synthesis of 2'-*N*-methyl neomycin **59**, the tetra-*N*-acetyl paromomycin intermediate **65** was converted to corresponding 2'-*N*-benzyl-*N*-methyl intermediate **78** with an overall 42% yield for the two steps. A two-step deprotection procedure, involving hydrogenolysis to cleave the benzyl group and saponification of acetamido groups in hot aqueous sodium hydroxide, and then purification on Sephadex and lyophilization from aqueous acetic acid afforded the 2'-*N*-methyl paromomycin **79** as per-acetate salt in 27% yield (Scheme 13). Reductive amination of **65** with acetaldehyde gave the 2'-*N*-ethyl intermediate **80** in 64% yield, which upon saponification in hot aqueous sodium hydroxide, and then purification on Sephadex and lyophilization from aqueous acetic acid afforded the 2'-*N*-ethyl paromomycin **81** as peracetate salt in 43% yield (Scheme 13). Similarly, reductive amination of **65** with propionaldehyde gave the 2'-*N*-propyl intermediate **82** in 70% yield, which upon saponification, and then purification on Sephadex and lyophilization from aqueous acetic acid gave the 2'-*N*-ethyl paromomycin **83** as peracetate salt in 49% yield (Scheme 13). The exact position of the alkyl groups was confirmed by heteronuclear multiple bond coherence (HMBC) correlations between the methyl and the 2'-position in the case of 2'-*N*-methyl paromomycin **79**, and between

the *N*-methylene and the 2'-position in the case of both 2'-*N*-ethyl paromomycin **81** and 2'-*N*-propyl paromomycin **83**.



Scheme 13. Synthesis of 2'-*N*-alkyl paromomycin derivatives **79**, **81** and **83**.

2'-Deoxy-2'-hydroxy paromomycin **86** was synthesized using the same *N,N*-dimethylformamide mediated α -selective glycosylation method applied for the synthesis of 2'-deoxy-2'-hydroxy neomycin B **70** (Scheme 10). The glycosylation reaction between thioglycoside donor **84**^{202,203} and glycosyl acceptor **67** gave the α -glycosylated product **85** in 46% yield (Scheme 14).¹⁹⁷ Finally, a two-step deprotection procedure, involving hydrogenolysis to deprotect the benzyl ethers, followed by saponification of the acetyl groups in hot aqueous sodium hydroxide, and then purification on Sephadex and lyophilization from aqueous acetic acid afforded the 2'-deamino-2'-hydroxy paromomycin **86** as the peracetate salt in 42% yield (Scheme 14).



Scheme 14. Synthesis of 2'-deamino-2'-hydroxy paromomycin **86**.

3.1.3. Biological evaluations:

The 2'-modified paromomycin derivatives **79**, **81**, **83**, and **86** were screened for their antiribosomal activities in cell free translational assays employing wild type bacterial ribosomes and recombinant hybrid bacterial ribosomes (Table 10). Their antiribosomal activities were also compared with those of the parent paromomycin **21**. The activity of paromomycin against the bacterial ribosome is not affected by 2'-*N*-alkylation (**79**, **81** and **83**, Table 10), which is consistent with the earlier reports on antibacterial activity of 2'-*N*-ethyl paromomycin¹⁸⁹ 2'-*N*-alkylated derivatives of sisomicin,¹⁸³⁻¹⁸⁸ and the results presented in section 2.2.3. on the 2'-*N*-alkylated neomycin B derivatives (**59** and **61**, Table 6). The 2'-deamino-2'-hydroxy paromomycin **86** on the other hand showed a 30-fold reduction in antiribosomal activity against the bacterial ribosome as compared to parent paromomycin (**86**, Table 10). This observation contrasts with the analogous replacement of the 2'-amino with a 2'-hydroxy group in neomycin B, which does not cause any change in antiribosomal activity towards bacterial A-site (**70**, Table 6). This difference between the paromomycin and neomycin series is attributed to the stronger A1408-6'NH₃⁺ hydrogen bond in neomycin that compensates for the loss of activity due to the replacement of 2'-amino group with a 2-hydroxy group, whereas the weaker A1408-6'OH hydrogen bond in the paromomycin-ribosome complex is less capable of doing this.⁶⁷

To compare the effect of each modification on ribosomal selectivity, the data presented in Table 10 have been converted into selectivity quotients (SelQ) with respect to the parent paromomycin as presented in Table 11. Similar to the 2'-*N*-alkylated derivatives of neomycin B, the 2'-*N*-alkyl modification of paromomycin increases selectivity for the bacterial over mutant mitochondrial ribosome to a greater extent than over the wild type mitochondrial ribosome (**79**, **81**, and **83**, Entries 1, 2 and 3, Table 7). It is interesting to note that the selectivity for bacterial

over the cytosolic ribosome constantly decreases as the size of the 2'-alkyl group increases. The 2'-deamino-2'-hydroxy modification is not helpful for increasing the selectivity of paromomycin (**86**, Entry 4, Table 11), whereas the analogous modification in neomycin B does increase the selectivity for the bacterial over mitochondrial and cytosolic ribosomes.

Table 10. Antiribosomal activities (IC₅₀, µg/mL) and selectivities of 2'-modified paromomycin derivatives (selectivities are obtained by dividing the eukaryotic values by the bacterial values).

Compound	In vitro <i>M. smegmatis</i> (IC ₅₀ , µg/mL)			
	Bacterial	Mit13 (Selectivity)	A1555G (Selectivity)	Cyt14 (Selectivity)
Paromomycin 21	0.04	47 (1175)	5 (125)	12 (300)
79	0.03	95 (3167)	34 (1133)	38 (1267)
81	0.03	141 (4700)	54 (1800)	27 (900)
83	0.05	153 (3060)	38 (760)	21 (420)
86	1.36	408 (300)	193 (142)	275 (202)

Table 11. Change in antiribosomal selectivity (selectivity quotient = selectivity of derivative/selectivity of parent) of 2'-modified derivatives with respect to parent paromomycin.

Entry	Compounds	Selectivity Quotient (SelQ)		
		Mit13	A1555G	Cyt14
1	79	2.7	9.06	4.22
2	81	4	14.4	3
3	83	2.6	6.08	1.4
4	86	0.25	1.14	0.67

All compounds were also screened for their antibacterial activities. The MIC data against the clinical isolates of the Gram-positive bacterium methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacterium *Escherichia coli* and *Pseudomonas aeruginosa* are presented in Table 12. The 2'-*N*-alkylated derivatives **79**, **81** and **83** showed comparable antibacterial activity to the parent paromomycin against MRSA and *E. coli*. The 2'-deoxy-2'-hydroxy derivative **86** is significantly less active as compared to paromomycin and 2'-*N*-alkylated derivatives, which is also reflected in the antiribosomal data (Table 12). Most of the compounds were found to be inactive against clinical isolates of the Gram-negative bacterium *Pseudomonas aeruginosa*.

Table 12. Antibacterial activities of 2'-modified paromomycin derivatives (MIC, µg/mL).

Compound	MRSA				<i>E. coli</i>			<i>P. aeruginosa</i>		
	AG038	AG039	AG042	AG044	AG001	AG055	AG003	AG031	AG032	AG033
Paromo. 21	4	>256	>256	4-8	16-32	8	8-16	>128	>128	>128
79	8	>128	>128	4	16	16	16	>128	>128	>128
81	8	>128	>128	4-8	16	16	16	>128	>128	>128
83	8-16	>128	>128	4	16	16	16	>128	>128	>128
86	64-128	>128	>128	64-128	>128	>128	>128	>128	>128	>128

These 2'-modified paromomycin derivatives were screened against wild type and recombinant *Escherichia coli* strains carrying defined resistance determinants in order to investigate their susceptibility to AAC (2') resistance mechanism (Table 13). The 2'-*N*-alkylated derivatives **79**, **81** and **83** retain their antibacterial activity and generally are not susceptible to modification by AAC (2') class of AMEs. The 2'-alkyl paromomycin derivatives are active against

E. coli strain carrying the ArmA gene and thus are not affected by the 16s-rRNA methyl transferase mechanism of resistance. The 2'-deoxy-2'-hydroxy derivative **86** does not show good antibacterial activity in general for wild type and resistance strain.

Table 13. Antibacterial data of 2'-modified paromomycin derivatives against wild type and engineered strains of *E. coli* carrying specific resistance determinants (MIC, µg/ml).

Strain:	AG006	AG106	pH434	pGB2
Resistance Mechanism:	Wild Type	AAC (2')-Ia	AAC (2')-Ib	ArmA
Paromomycin 21	1-2	>64	>64	2
79	2-4	4	8	4-8
81	2	-	4	4-8
83	2	-	4	4-8
86	32	-	-	-

3.1.4. Conclusion:

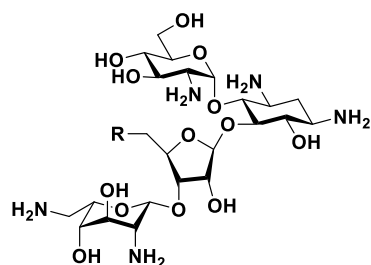
The 2'-*N*-alkylated paromomycin derivatives show comparable antibacterial activity to the parent paromomycin with moderately enhanced selectivity for the bacterial ribosome over mitochondrial and mutant mitochondrial ribosomes. These derivatives are not modified by the action of AAC-(2') mechanism of resistance and possess good antibacterial against the AAC-(2') resistant strains. The 2'-deoxy-2'-hydroxy modification of paromomycin shows significantly reduced antibacterial activity and selectivity.

3.2. 5''-modifications to paromomycin.

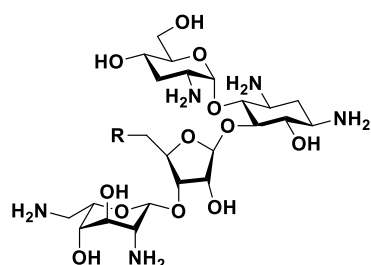
3.2.1. Introduction:

After modifying of the 2'-position in paromomycin, the 5'-position was targeted. In the decoding A-site the 5''-hydroxy group present in paromomycin, neomycin B and other members of 4,5-series of AGAs makes a hydrogen bond with N7 of the 1491G residue. It also makes an intramolecular hydrogen bond with the protonated 2'-amino group that is hypothesized to enforce correct conformation for binding with the bacterial A-site (Figures 11 and 20),^{135,177} albeit the data reported in the sections 2.2.3 and 3.1.3 in neomycin series do not support the need for this intramolecular hydrogen bond. Crucially, the 5''-hydroxy group is susceptible to modification by APH-(3', 5'') class of AMEs. These APH-(3', 5'') enzymes are capable of modifying both the 3' and/or 5''-hydroxy groups by attaching a negatively charged phosphate group and hence decreasing the antibacterial activity of AGAs.^{27,120,121} These enzymes cause a significant amount of resistance to a broad range of AGAs and thus represent a serious threat. Several attempts have been made in the past to modify the 5''-position in paromomycin and other 4,5-series AGAs to circumvent the action of these enzymes. Thus, the 5''-deoxy **87**, 5''-*O*-alkyl **88**, and 5''-fluoro **89** modifications of paromomycin are known to cause a significant loss of antibacterial activity (Figure 23).²⁰⁴ Similarly, 5''-deoxy lividomycin B **91** and 5''-fluoro lividomycin B **92** are eight fold less active than the parent lividomycin B **90**.²⁰⁵ The 5''-carboxylic acid **93**¹³⁵ and 5''-carboxamide **94**²⁰⁶ derivatives of paromomycin are inactive compounds (Figure 23). Cyclic 2',5''-anhydro derivatives of neomycin B and paromomycin having structures like **95** and **96** are also significantly less active than their parents (Figure 23).^{135,207} In contrast, 5''-deoxy-5''-amino derivatives of paromomycin and neomycin B (**97** and **98**) retain the antibacterial activity of their parents.²⁰⁸ The 5''-deoxy-5''-amino butirosin A **100** is more potent compound than the parent

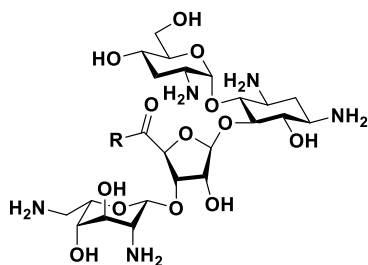
butironin A **99** (Figure 23).²⁰⁹ However, these 5''-deoxy-5''-amino modifications shows stronger affinity towards cytosolic ribosome and cause toxicity for cytosolic ribosomes, which forms the basis of the use of such compounds for the read through treatment of diseases like cystic fibrosis and the treatment of Leishmaniosis caused by protozoal parasites.¹⁷¹⁻¹⁷³ Some 5''-*O*-glycosylated neomycin B derivatives, especially the β -D-ribofuranoside **101** (Figure 23) show, comparable activity to the parent neomycin B and are poor substrate for APH-(3')-IIIa.¹⁴¹ Simple mono- and di-peptide derivatives like **102** of 5''-deoxy-5''-amino neomycin B **98** retain good antibacterial activity (Figure 23).¹⁴² Presumably, these 5''-amido derivatives have additional amino group and leading to the extra binding through electrostatic attraction. There are recent reports on neomycin B dimers like **103** and neomycin B-Anthroquinone conjugates like **104**, linked at 5''-position through urea and thiourea linkages and possess good antibacterial activity (Figure 24).^{146,210} Again, these compounds have additional polar functionalities and help for the binding. Overall, the literature precedence indicates that the presence of a hydrogen bond donor substituent at 5''-position is critical for binding. Based on these observations, the 5''-hydroxy group of paromomycin was modified to simple amide and urea type functionalities with the minimum unfunctionalized group tolerated, and their antiribosomal and antibacterial activities were screened.



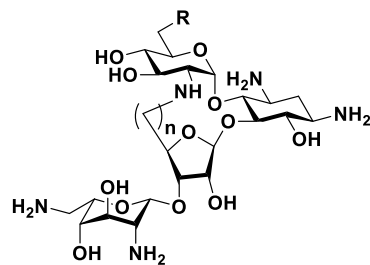
21, R = OH, Paromomycin
 87, R = H, 5''-Deoxy paromomycin
 88, R = alkoxy group, 5''-O-alkyl paromomycin
 89, R = F, 5''-Deoxy-5''-fluoro paromomycin



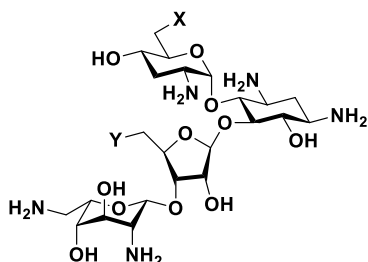
90, R = OH, Lividomycin B
 91, R = H, 5''-Deoxy lividomycin B
 92, R = F, 5''-Deoxy-5''-fluoro lividomycin B



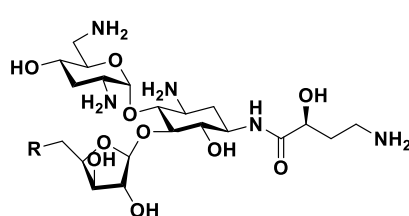
93, R = OH, Paromomycin-5''-carboxylic acid
 94, R = NH₂, Paromomycin-5''-carboxamide



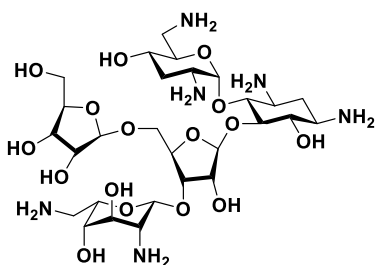
95, R = OH, 2',5''-Anhydro paromomycin
 96, R = NH₂, 2',5''-Anhydro neomycin B



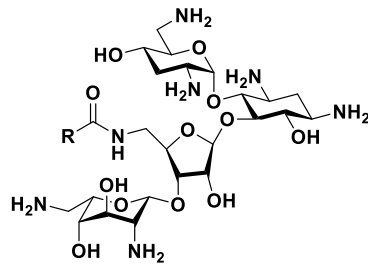
97, X = OH, Y = NH₂, 5''-Deoxy-5''-amino paromomycin
 98, X = Y = NH₂, 5''-Deoxy-5''-amino neomycin B



99, R = OH, Butirosin A
 100, R = NH₂, 5''-Deoxy-5''-amino butirosin A



101, 5''-O-β-D-ribofuranosyl neomycin B



R = CH₂NH₂, CH₂NH(CO)CH₂NH₂
 102, 5''-Deoxy-5''-amido neomycin B

Figure 23. Some of the 5''-modified derivatives of paromomycin, lividomycin B, neomycin B a neomycin B, and butirosin A.

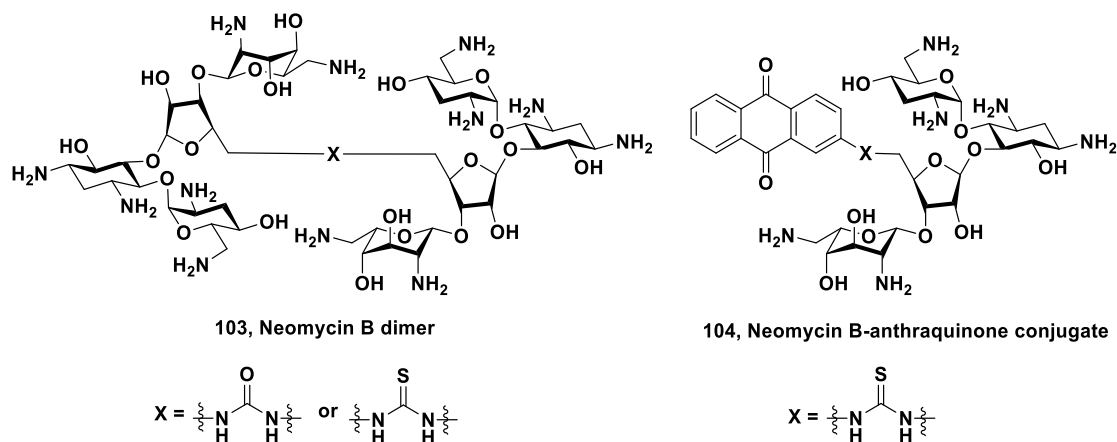
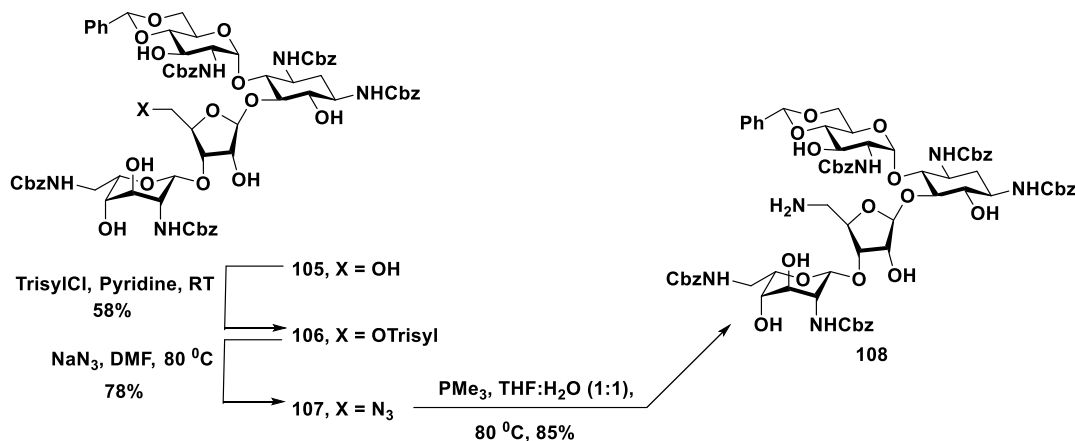


Figure 24. Neomycin B dimer and neomycin B-anthraquinone conjugated linked at 5''-position.

3.2.2. Synthesis:

The 5''-deoxy-5''-formamido, 5''-deoxy-5''-acetamido, and 5''-deoxy-5''-ureido paromomycin derivatives **110**, **112**, and **114** were prepared from intermediate **105**, which was readily obtained from paromomycin **21** by following the literature protocol.²¹¹ Selective sulfonylation of **105** with 2,4,6-triisopropylbenzenesulfonyl chloride gave **106** in 58% yield, which upon displacement with azide afforded 73% yield of **107**. Staudinger reaction to reduce the azide with trimethylphosphine in hot aqueous tetrahydrofuran afforded the 5''-deoxy-5''-amino intermediate **108**²¹¹ in 85% yield (Scheme 15). Treatment of **108** with formic acetic anhydride¹⁹¹ in dichloromethane gave the corresponding 5''-deoxy-5''-formamido intermediate **109** in 69% yield. Global deprotection by hydrogenolysis, followed by purification on Sephadex and lyophilization from aqueous acetic acid gave the 5''-deoxy-5''-formamido paromomycin **110** as peracetate salt in 30% yield (Scheme 16). For the synthesis of the 5''-deoxy-5''-acetamido analog **112**, treatment of the 5''-deoxy-5''-amino intermediate **108** with acetic anhydride in dichloromethane gave the corresponding 5''-deoxy-5''-acetamido intermediate along with the complete cleavage of the 4',6'-*O*-benzylidene acetal. Treatment with acetic anhydride in pyridine then gave the fully protected intermediate **111** with an overall 79% yield for the two steps. Finally,

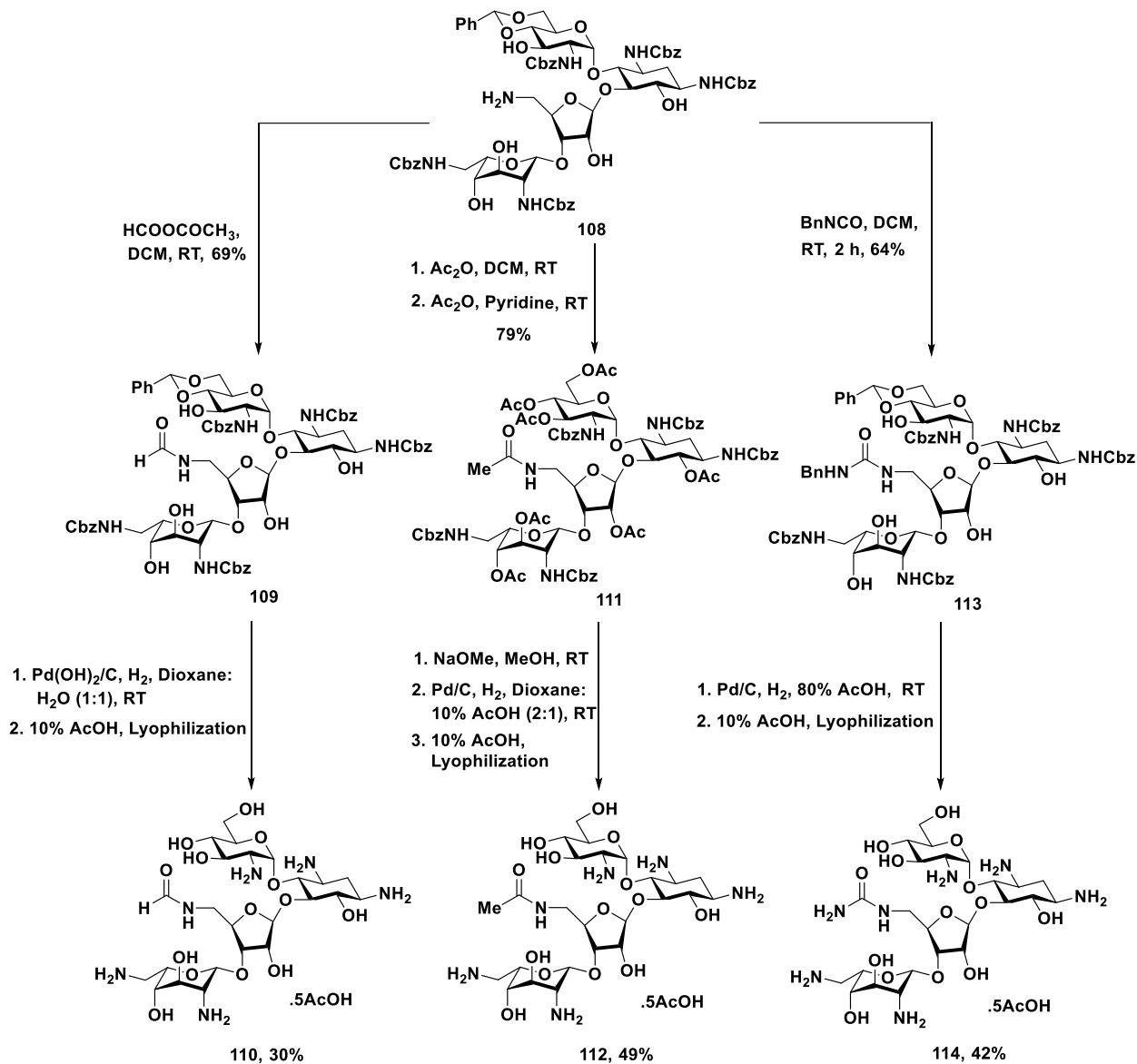
saponification of acetates by sodium methoxide in methanol, followed by hydrogenolysis to deprotect the benzyl carbamates, and then purification on sephadex and lyophilization from aqueous acetic acid gave 5''-deoxy-5''-acetamido paromomycin **112** as peracetate salt in 49% yield (Scheme 16). Treatment of the intermediate amine **108** with benzyl isocyanide in dichloromethane gave the corresponding benzyl protected 5''-deoxy-5''-ureido derivative **113** in 64% yield. Global deprotection by hydrogenolysis, followed by purification on Sephadex and lyophilization from aqueous acetic acid gave 5''-deoxy-5''-ureido paromomycin **114** as the peracetate salt in 42% yield (Scheme 16).



Scheme 15. Synthesis of 5''-deoxy-5''-amino intermediate **108**.

The rotation around the carbon-nitrogen bond in amides is restricted due to its partial double bond character, and because of this restricted rotation amides can exist either in the cis-configuration (E-configuration) or in the trans-configuration (Z-configuration). However, the trans-configurations predominates over the cis-configuration and this can be explained on the basis of steric interactions and the cis-configuration is destabilized by repulsion between R¹ and R² group (Figure 25).^{212,213} This preference for the trans-configuration has been shown by dipole moment and dielectric constant measurements and by ultraviolet, infrared, and Raman

spectroscopy.²¹⁴⁻²²² Similarly, the small ring lactams mostly exist in the cis-configurations, whereas in the large ring lactams the trans-configuration predominates over cis-configurations.^{223,224}



Scheme 16. Synthesis of 5''-modified paromomycin derivatives **110**, **112**, and **114**.

It was interesting to note that the 5''-deoxy-5''-formamido paromomycin **110** exhibited both cis- and trans-configurations. The relative integration of formyl hydrogen peak in the $^1\text{H-NMR}$ spectrum of 5''-deoxy-5''-formamido paromomycin **110** clearly indicated the presence of cis-conformer approximately in 13%. Whereas, the 5''-deoxy-5''-acetamido paromomycin **112** and 5''-deoxy-5''-ureido paromomycin **114** existed only in trans-configuration and did not show any indication of cis-configuration. Similar observations have been made in the literature and formamides have been reported to show both cis- and trans-conformers.^{212,213} The percentage of cis- and trans-conformers reported for *N*-methylformamide, *N*-ethylformamide, *N*-isopropylformamide, *N*-tert-butylformamide and the corresponding acetamides,²¹² and their comparison with the 5''-modified paromomycin derivatives are presented in Table 14. It is clear that none of the acetamides show the cis-conformer and this most probably due to the increased steric hindrance between the methyl group and the substituent present on the nitrogen atom. On the other hand, formyl hydrogen in formamides is sterically less cumbersome and consequently show significant amount of cis-conformer (Figure 25).

Table 14. Percentage of cis- and trans-conformers in various *N*-monosubstituted amides.

S.N.	Amide	% of Cis (E)	% of Trans (Z)
1.	<i>N</i> -Methylformamide	8	92
2.	<i>N</i> -Methylacetamide	0	100
3.	<i>N</i> -Ethylformamide	12	88
4.	<i>N</i> -Ethylacetamide	0	100
5.	<i>N</i> -Isopropylformamide	12	88
6.	<i>N</i> -Isopropylacetamide	0	100
7.	<i>N</i> -tert-Butylformamide	18	82
8.	<i>N</i> -tert-Butylacetamide	0	100
9.	5''-Deoxy-5''-formamido paromomycin 110	13	87

10.	5''-Deoxy-5''-acetamido paromomycin 112	0	100
11.	5''-Deoxy-5''-ureido paromomycin 114	0	100

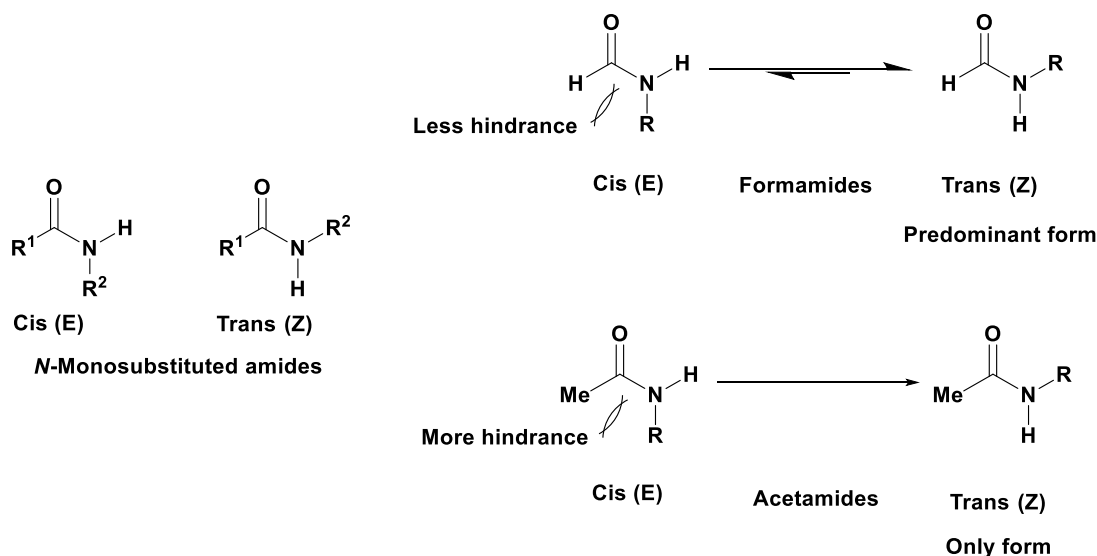


Figure 25. *Cis* (E)- and *trans* (Z)-configurations in *N*-monosubstituted amides.

3.2.3. Biological evaluations:

The 5''-modified paromomycin derivatives **110**, **112**, and **114** were screened for their antiribosomal activities in cell free translational assays employing wild type bacterial ribosomes and recombinant hybrid bacterial ribosomes (Table 14). Their antiribosomal activities were also compared with those of the parent paromomycin **21**. The 5''-formamido modification did not cause any reduction in the activity of paromomycin (**110**, Table 14). Presumably, the 5''-amidic NH can also serve as a hydrogen bond donor for the N7 of the G1491 residue. Consequently, the change from a 5''-hydroxy to a 5''-formamide does not have any significant effect on the binding affinity. Similarly, the 5''-ureido derivative **114** which also has a 5''-amidic NH group, possesses the comparable activity to the parent paromomycin. On the other hand, the 5''-acetamido derivative **112** showed an eight fold reduction in activity. A similar trend in loss of activity was

observed earlier in neomycin B series when going from 2'-*N*-formyl neomycin B to 2'-*N*-acetyl neomycin B (**76** and **77**, Table 6).

Table 15. Antiribosomal activities (IC₅₀, µg/mL) and selectivities of 5''-modified paromomycin derivatives (selectivities are obtained by dividing the eukaryotic values by the bacterial values).

Compound	In vitro <i>M. smegmatis</i> (IC ₅₀ , µg/mL)			
	Bacterial	Mit13 (Selectivity)	A1555G (Selectivity)	Cyt14 (Selectivity)
Paromomycin 21	0.04	47 (1175)	5 (125)	12 (300)
110	0.04	144 (3600)	30 (750)	90 (2250)
112	0.3	195 (650)	13 (43)	39 (130)
114	0.03	70 (2333)	28 (933)	89 (2967)

The antiribosomal activity data presented in Table 14 have been converted into selectivity quotients (SelQ) to compare the effect of each modification on ribosomal selectivity with respect to the parent paromomycin (Table 15). The 5''-formamido **110** and 5''-ureido **114** modifications of paromomycin showed a slight increase in selectivity for the bacterial over mitochondrial and mutant mitochondrial ribosome (Entries 1 and 3, Table 15). These two modifications also caused a moderate increase in selectivity for the bacterial over cytosolic ribosome (Entries 1 and 3, Table 15). The 5''-acetamido **112** derivative showed both reduced activity and selectivity (Entry 2, Table 15).

Table 16. Change in antiribosomal selectivity (selectivity quotient = selectivity of derivative/selectivity of parent) of 5''-modified derivatives with respect to parent paromomycin.

Entry	Compounds	Selectivity Quotient (SelQ)		
		Mit13	A1555G	Cyt14
1	110	3.06	6	7.5
2	112	0.55	0.34	0.43
3	114	1.98	7.46	7.42

The newly synthesized 5''-derivatives **110**, **112**, and **114** were screened against the clinical isolates of the Gram-positive bacterium methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacterium *Escherichia coli* and *Pseudomonas aeruginosa* in order to establish their antibacterial activity and the MIC data is presented in Table 16. The 5''-formamido **110** and 5''-ureido **114** modifications to paromomycin do not bring any significant change in antibacterial activities and have the similar antibacterial profile. The 5''-acetamido **112** modification generally caused a marked decrease in antibacterial activity against the clinical isolates of MRSA and *E. coli*. All the compounds including parent paromomycin are inactive against clinical isolates of *P. aeruginosa*.

The 5''-modified paromomycin derivatives **110**, **112**, and **114** were also screened against wild type and recombinant *Escherichia coli* strains carrying various APH-(3') resistance enzyme in order to validate the usefulness of these 5''- modifications in circumventing the APH-(3') mechanism of resistance (Table 17). As indicated by the data presented in Table 17, none of the compounds show a good antibacterial activity against the resistant strain carrying the APH-(3') resistance mechanism. This is because APH-(3') enzymes can modify both the 3' and 5''-hydroxy group by attaching a negatively charged phosphate group and hence decreasing the antibacterial activity of AGAs.^{27,120,121} Although in the present compounds the 5''-position is protected by

amide modifications, all still have a 3'-hydroxy substituent that is susceptible for modification by APH-(3') class of AMEs.

Table 17. Antibacterial activities of 5''-modified paromomycin derivatives (MIC, µg/mL).

Compound	MRSA				<i>E. coli</i>			<i>P. aeruginosa</i>		
	AG038	AG039	AG042	AG044	AG001	AG055	AG003	AG031	AG032	AG033
Paromo. 21	4	>256	>256	4-8	16-32	8	8-16	>128	>128	>128
110	4	>128	>128	2-4	4	4	4	≥128	128	>128
112	16-32	>128	>128	16	64	32	32-64	>128	>128	>128
114	4-8	>128	>128	4	16	16	16	>128	>128	>128

Table 18. Antibacterial data of 5''-modified paromomycin derivatives against wild type and engineered strains of *E. coli* carrying specific resistance determinants (MIC, µg/ml).

Strain:	pH430	pH421	pH422	AG037	pH423
Resistance Mechanism:	Wild Type	APH (3')-I	APH (3')-II	APH (3')-III	APH (3')-VI
Paromomycin 21	1-2	>128	>128	>128	>128
110	1-2	>64	>64	128	>64
112	8-16	>64	>64	-	>64
114	2-4	>64	>64	-	>64

3.2.4. Conclusion:

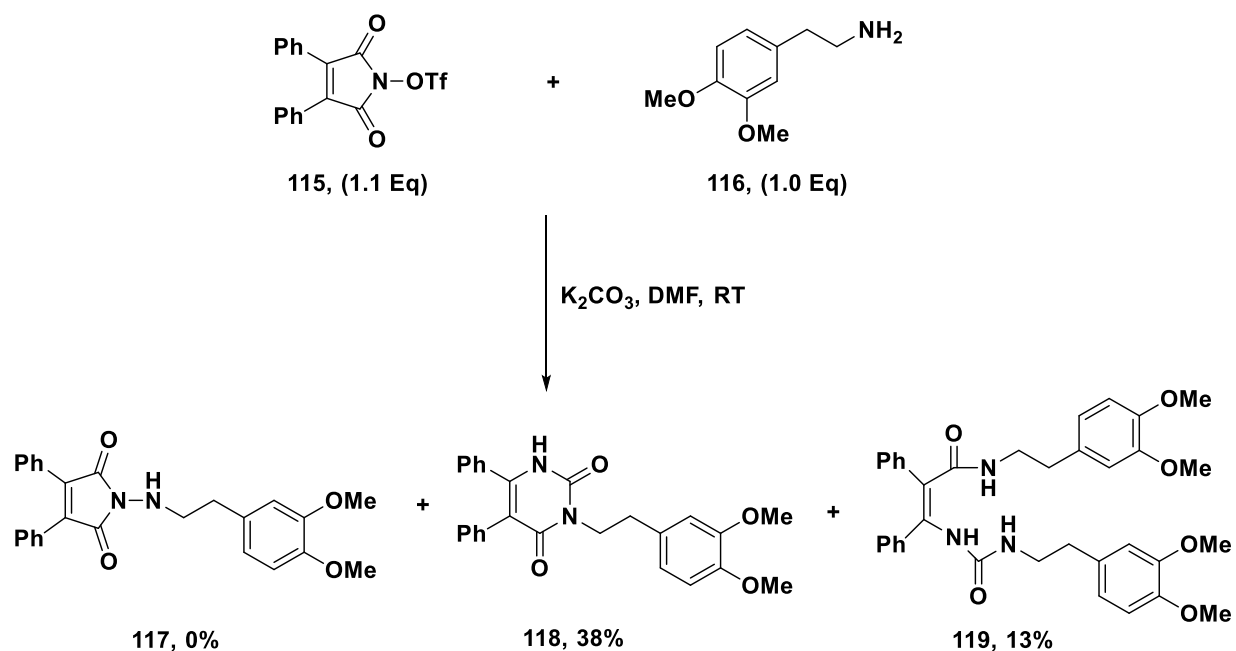
The 5''-formamido and 5''-ureido modifications does not cause any reduction in antibacterial activity of paromomycin and slightly increase the selectivity for the bacterial ribosome over mitochondrial, mutant mitochondrial and cytosolic ribosomes. The 5''-acetamido

derivative shows reduced antibacterial activity and selectivity. In combination with modifications at the 3'-position to subvert the effect of the AME at that position, the 5''-formamido and ureido modifications should provide compounds that are active in the presence of all classes of APH-(3',5'') enzymes. Such compounds are currently under investigation in the Crich laboratory.

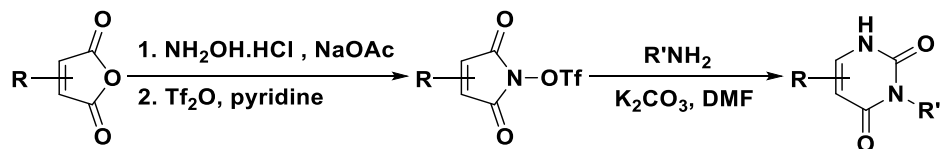
CHAPTER 4. SYNTHESIS OF 3-*N*-ALKYL PYRIMIDIN-2,4-DIONE DERIVATIVES.²²⁵

5.1. Introduction:

Nitrogen-based heterocycles are very commonly present in biologically active compounds and approved pharmaceuticals. The pyridines and pyrimidines are the most widespread nitrogen based heterocycles in approved drugs.²²⁶⁻²²⁸ The pyrimidin-2,4-dione derivatives are a very important class of compounds and are widespread in nature. They are one of the basic constituents of nucleic acids. The pyrimidin-2,4-dione ring structure is also found in various biologically active natural products.²²⁹ The medicinal properties of the pyrimidine-2,4-dione derivatives are well recognized and include antibacterial, antiviral, antineoplastic, antimalarial and antiparasitic activities.²²⁹ Therefore the development of new synthetic methods for substituted pyrimidine-based heterocycles is of inherent importance in drug discovery process. In the course of ongoing studies in our laboratory there was an occasion to react 2,3-diphenyl-*N*-trifloxymaleimide **115** with 3,4-dimethoxyphenylethylamine **116** in *N,N*-dimethylformamide in the presence of potassium carbonate at room temperature, with the goal and expectation of obtaining the maleimide-protected hydrazine derivative **117**. However, the unexpected major product was the pyrimidine-2,4-dione derivative **118**, which was formed together with the minor product amido urea **119** (Scheme 17). In this way, a new method was discovered for the synthesis of 3-*N*-alkyl pyrimidin-2,4-dione derivatives from readily available maleic anhydride via reaction of the derived *N*-trifloxymaleimides with amines. This novel two step entry into 3-*N*-alkyl pyrimidin-2,4-dione derivatives from *N*-hydroxymaleimides, themselves readily accessible the corresponding maleimides and hydroxylamine and primary amines is described in this chapter (Scheme 18).



Scheme 17. Unexpected formation of the 3-*N*-alkyl pyrimidin-2,4-dione derivative 118.

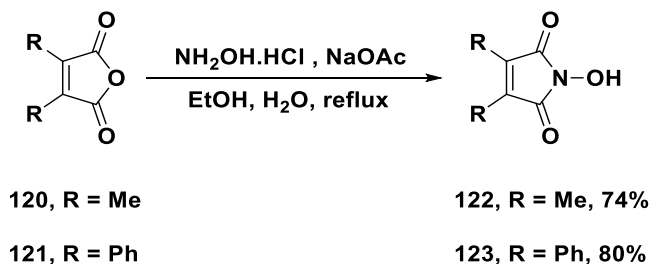


Scheme 18. Overall synthesis of the 3-*N*-alkyl pyrimidin-2,4-dione derivatives from maleic anhydrides.

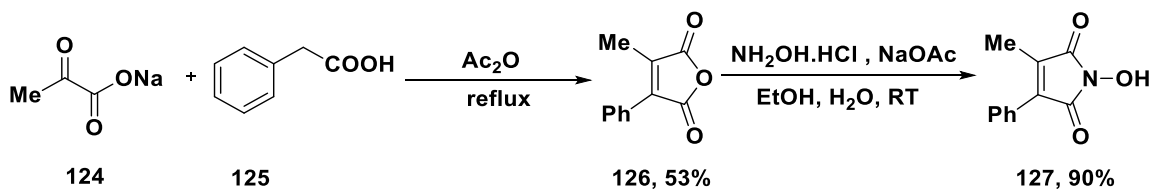
5.2. Synthesis:

Commercially available 2,3-dimethyl maleic anhydride **120** and 2,3-diphenyl maleic anhydride **121** were converted to the corresponding *N*-hydroxy maleimides **122** and **123** by refluxing with hydroxylamine hydrochloride and sodium acetate in a mixture of water and ethanol in 74% and 80% yields respectively (Scheme 19).²³⁰ The 2-methyl-3-phenyl maleic anhydride **126** was synthesized by refluxing sodium pyruvate **124** and phenyl acetic acid **125** in acetic anhydride in 53% yield.²³¹ Treatment of 2-methyl-3-phenyl maleic anhydride **126** with hydroxyl amine

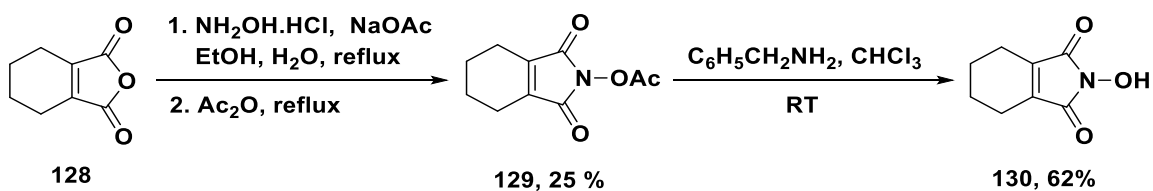
hydrochloride and sodium acetate in aqueous ethanol at room temperature afforded 2-methyl-3-phenyl-*N*-hydroxy maleimide **127** in 90% yield (Scheme 20). Treatment of commercial cyclohexene-1,2-dicarboxylic anhydride **128** with hydroxylamine hydrochloride and sodium acetate in hot mixture of water and ethanol gave the intermediate hydroxamic acid, which upon refluxing in acetic anhydride cyclized to the *N*-acetoxy imide **129** with an overall 25% yield for the two steps.^{230,232} The acetyl group was deprotected using benzylamine in chloroform²³² to give the *N*-hydroxyimide derivative **130**²³³ in 62% yield (Scheme 21). All the *N*-hydroxymaleimides **122**, **123**, **127**, and **130** were converted to the corresponding *N*-trifloxymaleimides **131**, **115**, **132**, and **133** by treatment with triflic anhydride in the presence of pyridine in dichloromethane (Scheme 22).



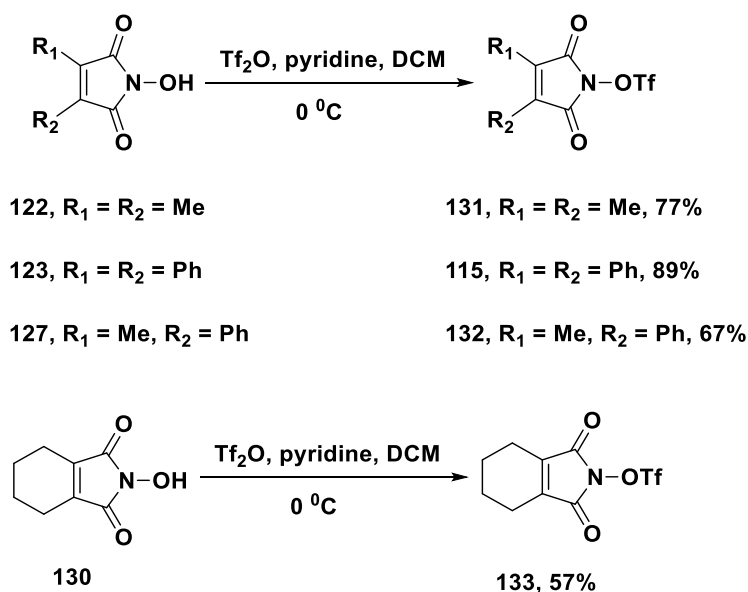
Scheme 19. Synthesis of *N*-hydroxy maleimide derivatives **122** and **123**.



Scheme 20. Synthesis of *N*-hydroxymaleimide derivative **127**.

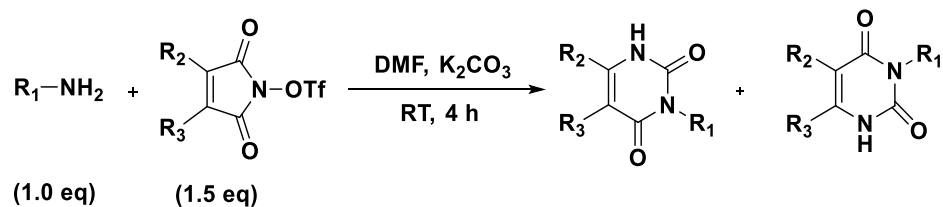


Scheme 21. Synthesis of *N*-hydroxymaleimide derivative **130**.



Scheme 22. Synthesis of *N*-trifloxymaleimide derivatives **131**, **115**, **132**, and **133**.

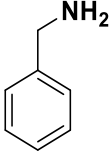
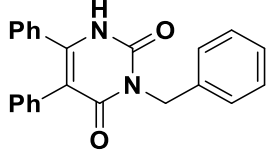
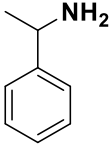
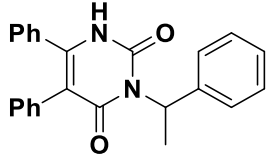
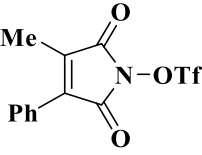
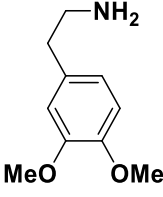
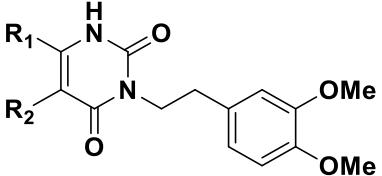
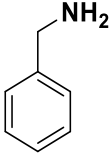
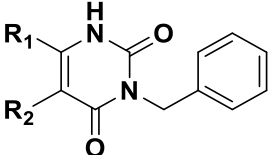
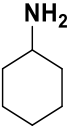
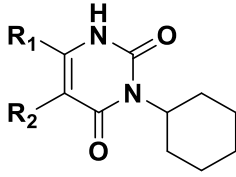
For the crucial reaction forming the 3-*N*-alkyl pyrimidin-2,4-dione derivatives, a general reaction protocol was established. According to this reaction protocol, a stirred suspension of *N*-trifloxymaleimide derivative and potassium carbonate in *N,N*-dimethylformamide was treated with a solution of primary amine in *N,N*-dimethylformamide at room temperature and then stirred for 4 hours at room temperature (Scheme 23). By following the same reaction protocol a series of 3-*N*-alkyl pyrimidin-2,4-dione derivatives were synthesized from the corresponding *N*-trifloxy maleimides and primary amines with the results are summarized in Table 18.

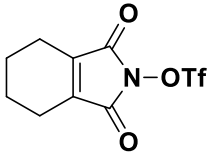
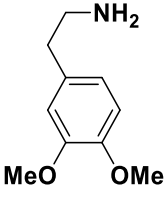
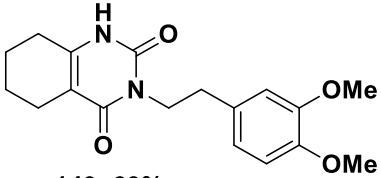
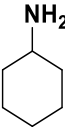
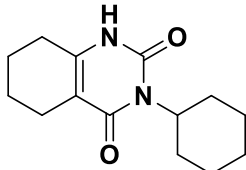


Scheme 23. Common reaction procedure for the synthesis of 3-*N*-alkyl pyrimidin-2,4-dione derivatives.

Table 19. Examples of synthesized 3-*N*-alkyl pyrimidin-2,4-dione derivatives.

Entry	<i>N</i> -trifloxy imide	Amine	Pyrimidindione, % yield
1	 131	 134	 138, 53 %
2		 135	 139, 56 %
3		 136	 140, 40 %
4	 115	 116	 118, 51 %

5		 <p>137</p>	 <p>141, 54 %</p>
6		 <p>136</p>	 <p>142, 16 %</p>
7	 <p>132</p>	 <p>116</p>	 <p>143: R₁ = Me, R₂ = Ph 144: R₁ = Ph, R₂ = Me 143 + 144 = 54% 143:144 = 1.2:1</p>
8		 <p>137</p>	 <p>145: R₁ = Me, R₂ = Ph 146: R₁ = Ph, R₂ = Me 145 + 146 = 51% 145:146 = 1.5:1</p>
9		 <p>135</p>	 <p>147: R₁ = Me, R₂ = Ph 148: R₁ = Ph, R₂ = Me 147 + 148 = 35% 147:148 = 1.5:1</p>

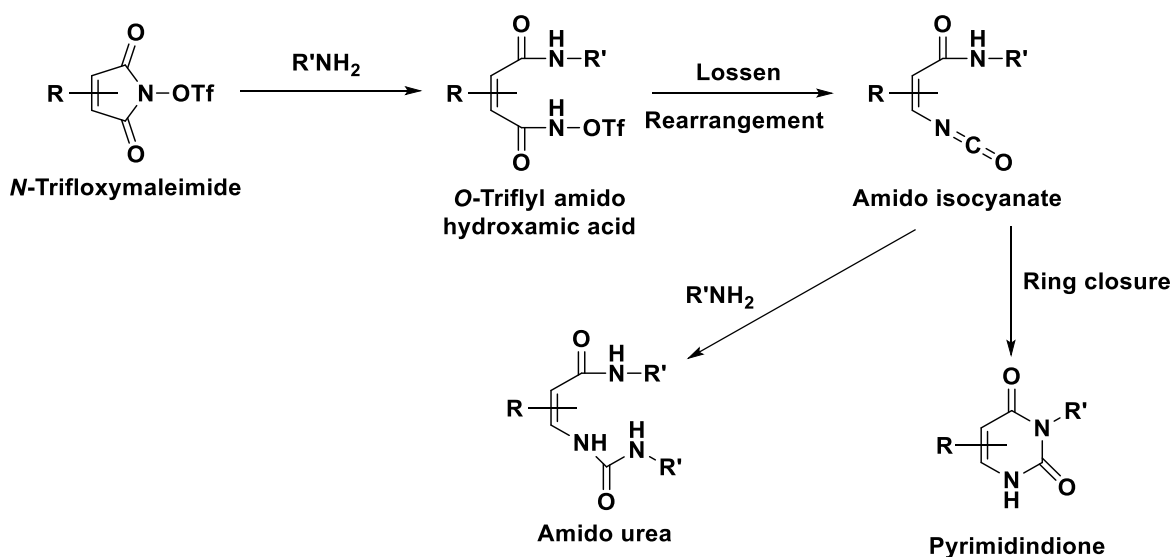
10	 <p>133</p>	 <p>116</p>	 <p>149, 63%</p>
11		 <p>135</p>	 <p>150, 55 %</p>

5.3. Discussion:

The examples shown in Table 18 demonstrate that the reaction methodology works smoothly to give the variously substituted 3-*N*-alkyl pyrimidin-2,4-dione derivatives in moderate to good yields. The method functions well with a variety of 2,3-disubstituted *N*-hydroxymaleimide derivatives including dimethyl (Entries 1-3), diphenyl (Entries 4-6), mixed methyl, phenyl (Entries 7-9), and fused bicyclic system (Entries 10 and 11). The synthesized 3-*N*-alkyl pyrimidin-2,4-dione derivatives also demonstrate the successful incorporation of variety of primary amines with different steric environments and functionalities. As expected, in the case of unsymmetrical 2-methyl-3-phenyl-*N*-trifloxymaleimide **132** two regioisomeric products were formed (Entries 7-9, Table 18). There was a modest preference for the formation of the isomer coming from the nucleophilic attack by the amine to the carbonyl carbon adjacent to the larger substituent. This modest preference for the nucleophilic attack to the carbonyl carbon adjacent to the larger substituent is due to the lesser steric hindrance for the approach of the nucleophile along the Burgi-Dunitz trajectory. Similar regioselectivities were observed in the past for the metal hydride reductions of 2,2-dimethylsuccinic anhydride and 2,2-dimethyl succinimide.^{234,235} The formation of 3-*N*-alkyl pyrimidin-2,4-diones was also accompanied with the formation of typical amido urea

byproducts, although their formation can be minimized to an extent by using a slight excess of electrophilic *N*-trifloxymaleimides as compared to the amine during the reaction.

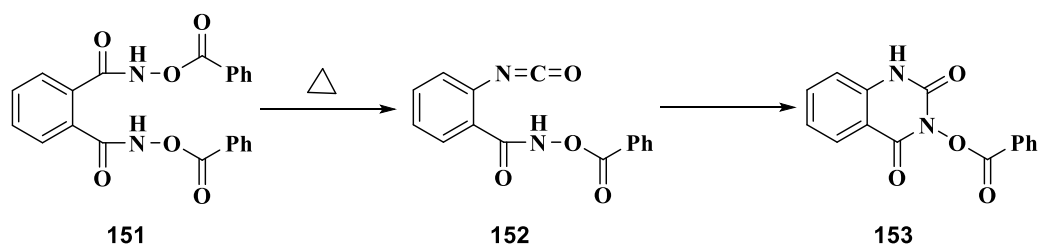
Mechanistically, the formation of 3-*N*-alkyl pyrimidin-2,4-diones involve the nucleophilic attack by the amine to one of the carbonyl carbons of the *N*-trifloxymaleimide to give an *O*-triflyl amido hydroxamic acid intermediate. This hydroxamic acid derivative undergoes rapid Lossen rearrangement^{236,237} to give an amido isocyanate. Finally, intramolecular nucleophilic attack of the amidic nitrogen atom to the isocyanate produces the major product 3-*N*-alkyl pyrimidin-2,4-dione. External nucleophilic attack by the amine on the amido isocyanate produces the amido urea byproducts (Scheme 23).



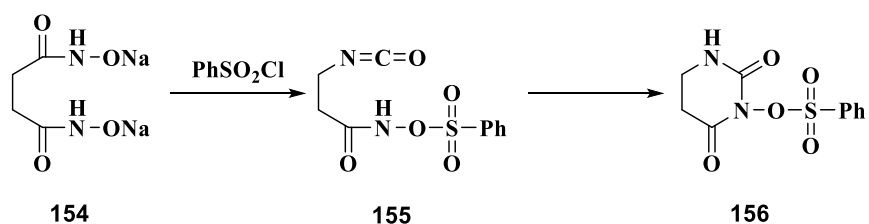
Scheme 24. Reaction mechanism for the formation of the 3-*N*-alkyl pyrimidin-2,4-dione.

The proposed reaction mechanism for the formation of the 3-*N*-alkyl pyrimidin-2,4-dione is also supported by literature precedent. Thus, Hurd and coworkers reported formation of 3-*N*-benzoyloxy quinazolinedione **153** by refluxing the disodium salt of *O,O'*-dibenzoyl phthalohydroxamic acid **151** in water. This process was considered to involve Lossen rearrangement of a hydroxamic acid derivative to give an isocyanate **152** which was

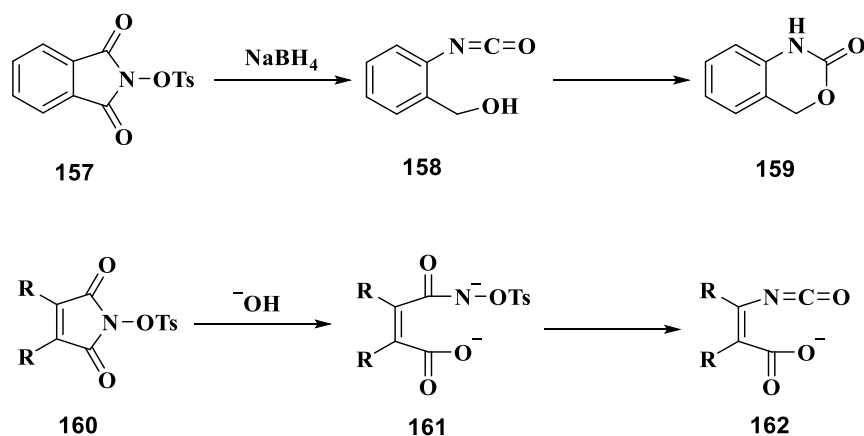
intramolecularly trapped by nucleophilic attack of the second hydroxamate (Scheme 25).²³⁸ Hurd and coworkers also reported Lossen rearrangement of sodium succinohydroxamate **154** on reaction with benzenesulfonyl chloride to afford 3-*N*-benzenesulfonyloxy dihydrouacil **156** via the isocyanate intermediate **155** (Scheme 26).²³⁹ Nucleophilic ring opening of *N*-toluenesulfonyloxy phthalimide **157** and maleimide derivative **160** by either borohydride or hydroxide followed by Lossen rearrangement of the intermediate *N*-toluenesulfonyloxy hydroxamic acid derivative was also reported by Barton and coworkers (Scheme 27).^{232,240}



Scheme 25. Formation of 3-*N*-benzoyloxy quinazolinedione **151**.



Scheme 26. Formation of 3-*N*-benzenesulfonyloxy dihydrouacil **154**.



Scheme 27. Lossen rearrangement of *N*-toluenesulfonyloxy phthalimide **157** and maleimide derivative **160**.

5.4. Conclusion:

In conclusion, an efficient and facile method was developed for the synthesis of highly substituted pyrimidin-2,4-dione derivatives. This is a novel two step entry into a very important heterocyclic scaffold pyrimidin-2,4-dione, from easily accessible and inexpensive *N*-hydroxy maleimides and primary amines. This synthetic method compliments and extends the existing routes for the synthesis of pyrimidine derivatives and the use of cyclic anhydrides²⁴¹ and thioanhydrides²⁴²⁻²⁴⁵ in multicomponent reactions.

CHAPTER 5. OVERALL CONCLUSIONS.

With a goal of developing a new generation of resistance-proof and less ototoxic aminoglycoside antibiotics, the 4,5- series AGAs neomycin B and paromomycin were selected for modifications. A series of individual modifications were made to these AGAs, and their influence on activity and selectivity was determined by antiribosomal activity in cell free translation assays. In order to determine their antibacterial activity, the compounds were screened against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* and *Pseudomonas aeruginosa*. They were also screened against genetically engineered *Escherichia coli* strains carrying specific resistance enzymes to investigate if these modifications are helpful in circumventing modification by common AMEs. Such studies of individual modifications permit the informed design of advanced compounds incorporating multiple modifications and should ultimately lead to the resistance proof and less ototoxic compounds.

In the neomycin B series, the most important modifications were made at the 6'-, 4'-, and 2'-positions. The 6'-*N*-(2-hydroxyethyl) modification brings a moderate increase in selectivity without any reduction in activity. Moreover, the compound showed strong activity in the presence of the AAC-(6') mechanism of resistance. The 4'-*O*-ethyl modification did not cause any reduction in antibacterial activity and effectively blocked the action of ANT-(4', 4''). However, combination of 6'-*N*-(2-hydroxyethylation) and 4'-*O*-ethylation caused a significant decrease in activity without much improvement in selectivity. Modifications at the 2'-positions showed the most encouraging results. The 2'-*N*-alkyl, 2'-deamino, and 2-deamino-2'-hydroxy modifications did not cause any reduction in activity and moderately increased the selectivity. These modifications were able to circumvent the AAC-(2') mechanism of resistance. Interestingly, the 2'-deamono neomycin B was equally active as the parent neomycin B, which invalidates the hypothesis that

the 2'-amino group is necessary to enforce the correct conformation for binding through internal hydrogen bonding with 5''-hydroxy group. Unfortunately, simple amide type modifications of 2'-amino group caused a significant decrease in activity.

In the paromomycin series, the 2'- and 5''-positions were selected for modification. The 2'-*N*-alkylated paromomycin derivatives showed comparable activity to paromomycin with a slight increase in selectivity. These 2'-*N*-alkylated derivatives were able to evade modification by the AAC-(2') class of AMEs. These results also demonstrate that the bacterial ribosomal A-site can accommodate at least a three carbon substituent at this position. The 2'-deamino-2'-hydroxy paromomycin derivative showed a thirty fold decrease in activity, whereas the identical modification in neomycin B did not cause any change in activity. This observation clearly demonstrates the difference between paromomycin and neomycin B. In neomycin B, the stronger A1408-6'NH₃⁺ hydrogen bond compensates for the loss of activity, whereas the weaker A1408-6'OH hydrogen bond in paromomycin is less capable of doing this. The 5''-deoxy-5''-formamido and 5''-deoxy-5''-ureido derivatives did not show any reduction in activity, constituting one of the most important findings of this project. Presumably, the 5''-amidic NH can also make the crucial interaction with the G1491 residue through hydrogen bonding. Combination of these 5''-modifications with modifications at 3'-position should give the compounds which completely evade the action of APH-(3',5'') class of AMEs. The 5''-acetamido derivative shows reduced antibacterial activity and selectivity.

The results obtained in the neomycin B and paromomycin series clearly show that there are several advantageous modification has been made and some compounds show strong antibacterial activity even in the presence of common AMEs with improved selectivity. Overall, these results demonstrate that it is possible to develop the new generation of resistance-proof and less ototoxic

AGAs by rational design.

In addition to the synthesis of novel AGAs, a new and facile method for the synthesis of 3-*N*-alkyl pyrimidin-2,4-dione derivatives was developed. The pyrimidinediones are one of the important class of nitrogen based heterocycles and commonly found in biologically active compounds and pharmaceutical agents. This synthetic method provides an easy access to highly substituted pyrimidine-2,4-dione derivatives from easily available and inexpensive maleic anhydrides and primary amines.

CHAPTER 6. EXPERIMENTAL SECTION.

General Experimental: All experiments were carried out under a dry argon atmosphere unless otherwise specified. All reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise specified. Chromatographic purifications were carried over silica gel (230-400 mesh). Thin layer chromatography was performed with pre-coated glass backed plates (w/UV 254). TLC were visualized by UV irradiation (254 nm) and by charring with sulfuric acid in ethanol (20:80, v/v) or ceric ammonium molybdate solution [$\text{Ce}(\text{SO}_4)_2$: 4 g, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$: 10 g, H_2SO_4 : 40 mL, H_2O : 360 mL]. Reversed-phase HPLC was operated by using Varian Star Chromatography Workstation software (version 6) with Varian HPLC equipment with two PrepStar model 218 pumps, a ProStar model 330 diode array detector and a ProStar model 701 fraction collector. Optical rotations were measured at 589 nm and 23 °C on an Autopol III polarimeter (Rudolph Research Analytical, Hackettstown, NJ) with a path length of 10 cm. Melting points are not corrected. ^1H and ^{13}C NMR spectra of all compounds were recorded using 400 and 600 MHz instrument. ESIHRMS were recorded using a Waters LCT Premier Xe TOF mass spectrometer.

1,3,2',2'',6'''-Pentazido-6,3',2'',5'',3'',4'''-hexa-*O*-benzyl-6'-*O*-(2,4,6-triisopropylbenzene sulfonyl)- 1,3,2',2'',6'''-pentadeamino paromomycin (24). A stirred solution of compound **23** (550 mg, 0.43 mmol) in DCM (2.7 mL) was treated with triethyl amine (0.48 mL, 3.42 mmol) at RT and 2,4,6-triisopropylbenzenesulfonyl chloride (388 mg, 1.28 mmol) was added while stirring. After stirring for 24 h at RT the reaction was quenched with water (20 mL). The DCM layer was separated and water layer was extracted with DCM (2 x 20 mL). The combined organic layers were washed with water, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with

ethyl acetate/hexane (1:4) to give the desired product **24** (350 mg, 53%). $[\alpha]_{\text{D}}^{\text{RT}} +65.7$ (*c* 2.4, CHCl_3). ^1H NMR (600 MHz, CDCl_3) δ 7.47 – 7.02 (m, 32 H), 6.14 (d, $J = 3.5$ Hz, 1H), 5.67 (d, $J = 5.9$ Hz, 1H), 4.99 (d, $J = 10.6$ Hz, 1H), 4.91 – 4.89 (m, 2H), 4.70 (d, $J = 10.8$ Hz, 1H), 4.68 (d, $J = 11.4$ Hz, 1H), 4.63 (d, $J = 11.8$ Hz, 2H), 4.50 (d, $J = 9.3$ Hz, 2H), 4.46 (d, $J = 11.9$ Hz, 1H), 4.43 (d, $J = 12.0$ Hz, 1H), 4.33 (d, $J = 12.0$ Hz, 1H), 4.30 – 4.21 (m, 5H), 4.18 (sep, $J = 6.7$ Hz, 2H), 4.15 – 4.10 (m, 2H), 3.87 (d, $J = 9.5$ Hz, 1H), 3.82 (dd, $J_1 = 10.1$ Hz, $J_2 = 1.3$ Hz, 1H), 3.80 – 3.75 (m, 2H), 3.69 – 3.62 (m, 2H), 3.57 (dd, $J_1 = 10.3$ Hz, $J_2 = 2.9$ Hz, 1H), 3.47 – 3.34 (m, 4H), 3.29 (t, $J = 9.4$ Hz, 1H), 3.13 (br s, 1H), 2.95 – 2.87 (m, 2H), 2.85 (dd, $J_1 = 10.3$ Hz, $J_2 = 3.6$ Hz, 1H), 2.26 (d, $J = 3.6$ Hz, 1H), 2.22 (dt, $J_1 = 13.0$ Hz, $J_2 = 4.5$ Hz, 1H), 1.38 (q, $J = 12.9$ Hz, 1H), 1.29 (d, $J = 6.8$ Hz, 12 H), 1.27 – 1.25 (m, 6 H). ^{13}C NMR (150 MHz, CDCl_3) δ 153.8, 150.9, 138.1, 138.0, 137.9, 137.6, 137.0, 136.9, 129.2, 128.7, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.5, 127.4, 127.2, 123.8, 106.0, 98.7, 96.0, 84.2, 82.5, 82.07, 81.83, 79.5, 75.6, 75.1, 74.9, 74.6, 74.3, 73.4, 73.2, 72.9, 72.4, 71.7, 71.5, 70.1, 70.0, 69.9, 68.0, 62.4, 60.4, 60.1, 57.3, 51.1, 34.2, 32.5, 29.7, 24.9, 24.8, 23.5. ESIHRMS calculated for $\text{C}_{80}\text{H}_{93}\text{N}_{15}\text{O}_{16}\text{SNa}$ $[\text{M}+\text{Na}]^+$, 1574.6543; found, 1574.6583.

1,3,2',6',2'',6'''-Hexaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-1,3,2',6',2'',6'''-hexa deamino neomycin B (25). To a stirred solution of **24** (260 mg, 0.17 mmol) in DMF (2.6 mL) was added sodium azide (109 mg, 1.67 mmol). The reaction mixture was stirred for 3 h at 80 $^{\circ}\text{C}$ then cooled to RT and quenched with water (10 mL). The water layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with water followed by brine, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with ethyl acetate/hexane (1:3) to give the desired product **25** (160 mg, 73%). $[\alpha]_{\text{D}}^{\text{RT}} +75.9$ (*c* 1.45, CHCl_3). ^1H NMR (600 MHz, CDCl_3) δ 7.55 – 6.99 (m, 30

H), 6.22 (d, $J = 3.4$ Hz, 1H), 5.70 (d, $J = 5.8$ Hz, 1H), 5.06 – 4.84 (m, 3H), 4.73 (d, $J = 10.6$ Hz, 1H), 4.66 – 4.60 (m, 3H), 4.56 – 4.45 (m, 3H), 4.43 (d, $J = 11.9$ Hz, 1H), 4.33 (d, $J = 12.0$ Hz, 1H), 4.33 – 4.26 (m, 2H), 4.26 (d, $J = 12.0$ Hz, 1H), 4.09 – 4.02 (m, 1H), 4.01 – 3.94 (m, 2 H), 3.90 – 3.74 (m, 4H), 3.74 – 3.63 (m, 2H), 3.58 (dd, $J_1 = 10.1$ Hz, $J_2 = 2.8$ Hz, 1H), 3.52 – 3.40 (m, 3H), 3.39 – 3.28 (m, 3H), 3.24 (t, $J = 9.3$ Hz, 1H), 3.13 (br s, 1H), 2.95 – 2.83 (m, 2H), 2.24 (dt, $J_1 = 12.8$ Hz, $J_2 = 4.2$ Hz, 1H), 1.43 (q, $J = 12.8$ Hz, 1H). ^{13}C NMR (150 MHz, CDCl_3) δ 138.2, 137.9, 137.9, 137.6, 137.0, 136.9, 128.8, 128.7, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 127.8, 127.8, 127.8, 127.5, 127.3, 106.1, 98.6, 95.8, 84.3, 82.5, 82.1, 81.7, 79.7, 75.6, 75.1, 75.0, 74.9, 74.4, 73.3, 73.3, 72.9, 72.4, 71.7, 71.5, 71.2, 71.0, 70.2, 62.6, 60.4, 60.2, 57.2, 51.6, 51.1, 32.6. ESIHRMS calculated for $\text{C}_{65}\text{H}_{70}\text{N}_{18}\text{O}_{13}\text{Na}$ $[\text{M}+\text{Na}]^+$, 1333.5267; found, 1333.5288.

1,3,2',6',2'',6'''-Hexaazido-6,3',2'',5'',3'',4'''-hexa-*O*-benzyl-4'-*O*-ethyl-

1,3,2',6',2'',6'''-hexadeamino neomycin B (26). To a stirred suspension of sodium hydride (10 mg, 0.24 mmol) in DMF (0.3 mL) was added a solution of **25** (160 mg, 0.12 mmol) in DMF (1.3 mL) at 0 °C. After stirring for 3 h at 0 °C the reaction was quenched with water (10 mL). The water layer was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with water followed by brine, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with ethyl acetate/hexane (1:4) to give the desired product **26** (120 mg, 73%). $[\alpha]_{\text{D}}^{\text{RT}} +86.3$ (c 1.71, CHCl_3). ^1H NMR (600 MHz, CDCl_3) δ 7.47 – 7.12 (m, 30H), 6.17 (d, $J = 3.7$ Hz, 1H), 5.69 (d, $J = 5.7$ Hz, 1H), 4.98 (d, $J = 10.7$ Hz, 1H), 4.93 (d, $J = 1.5$ Hz, 1H), 4.87 – 4.78 (m, 2H), 4.74 (d, $J = 10.7$ Hz, 1H), 4.64 (d, $J = 12.3$ Hz, 1H), 4.62 (d, $J = 12.3$ Hz, 1H), 4.56 (d, $J = 11.9$ Hz, 1H), 4.48 (dd, $J_1 = 11.8$ Hz, $J_2 = 4.2$ Hz, 2H), 4.44 (d, $J = 12.0$ Hz, 1H), 4.34 (d, $J = 12.0$ Hz, 1H), 4.32 – 4.25 (m, 3H), 4.09 (ddd, $J_1 = 8.2$ Hz, $J_2 = 5.7$ Hz, $J_3 = 2.1$ Hz, 1H), 3.98 (t, $J = 5.2$ Hz, 1H), 3.97 – 3.92 (m, 2H), 3.85 – 3.76 (m,

4H), 3.72 – 3.64 (m, 2H), 3.62 – 3.55 (m, 2H), 3.52 (dd, $J_1 = 13.0$, $J_2 = 2.1$ Hz, 1H), 3.50 – 3.41 (m, 2H), 3.40 – 3.31 (m, 3H), 3.15 (br s, 1H), 3.08 (t, $J = 9.4$ Hz, 1H), 3.00 (dd, $J_1 = 10.4$ Hz, $J_2 = 3.7$ Hz, 1H), 2.90 (dd, $J_1 = 12.9$ Hz, $J_2 = 3.9$ Hz, 1H), 2.25 (dt, $J_1 = 13.0$ Hz, $J_2 = 4.4$ Hz, 1H), 1.45 (q, $J = 12.8$ Hz, 1H), 1.18 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (150 MHz, CDCl_3) δ 138.3, 138.1, 137.9, 137.7, 137.0, 137.0, 128.7, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 127.8, 127.8, 127.8, 127.5, 106.2, 98.6, 95.6, 84.2, 82.5, 82.1, 81.7, 79.7, 79.1, 77.3, 77.1, 76.9, 75.6, 75.3, 75.1, 74.4, 73.3, 72.9, 72.4, 71.7, 71.50, 71.1, 70.2, 68.5, 63.1, 60.4, 60.0, 57.3, 51.4, 51.1, 32.5, 15.7. ESIHRMS calculated for $\text{C}_{67}\text{H}_{74}\text{N}_{18}\text{O}_{13}\text{Na}$ $[\text{M}+\text{Na}]^+$, 1361.5580; found, 1361.5559.

4'-O-Ethyl neomycin B acetate salt (27). To a stirred suspension of Pd/C (240 mg) in 10% AcOH (1.5 mL) was added a solution of **26** (120 mg, 0.09 mmol) in dioxane (1.5 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (45 psi) for 24 h, filtered, concentrated and purified by Sephadex C-25 column chromatography (1.0% ammonium hydroxide). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to give the desired product **27** as the peracetate salt (37 mg, 41%). $[\alpha]_{\text{D}}^{\text{RT}} +46.3$ (c 1.06, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.76 (d, $J = 3.7$ Hz, 1H), 5.14 (d, $J = 2.1$ Hz, 1H), 5.01 (d, $J = 1.2$ Hz, 1H), 4.21 (t, $J = 5.7$ Hz, 1H), 4.11 (dd, $J_1 = 4.6$ Hz, $J_2 = 2.4$ Hz, 1H), 4.03 (t, $J = 4.4$ Hz, 1H), 3.92 – (m, 2H), 3.82 (t, $J = 10.7$ Hz, 1H), 3.80 (t, $J = 10.3$ Hz, 1H), 3.65 (t, $J = 7.8$ Hz, 1H), 3.65 – 3.56 (m, 3H), 3.54 (br s, 1H), 3.50 – 3.43 (m, 2H), 3.41 (t, $J = 9.9$ Hz, 1H), 3.30 (br s, 1H), 3.25 – 3.11 (m, 4H), 3.11 – 2.95 (m, 3H), 2.20 (dt, $J_1 = 12.5$ Hz, $J_2 = 4.2$ Hz, 2H), 1.75 (s, 18H), 1.61 (q, $J = 12.6$ Hz, 1H) 0.90 (t, $J = 7.0$ Hz, 2H). ^{13}C NMR (150 MHz, D_2O) δ 178.0, 109.9, 95.2, 94.7, 84.6, 81.4, 78.5, 75.1, 75.0, 73.4, 72.2, 70.0, 69.1, 68.9, 67.8, 67.4, 67.1, 59.9, 53.1, 50.7, 49.6, 48.2, 40.2, 39.9, 27.8, 21.3, 14.5. ESIHRMS calculated for $\text{C}_{25}\text{H}_{51}\text{N}_6\text{O}_{13}$ $[\text{M}+\text{H}]^+$, 643.3514; found, 643.3500.

6'-N-Benzylloxycarbonyl neomycin B (28), 6'''-N-Benzylloxycarbonyl neomycin B (29) and 6',6'''-Bis-N-(benzylloxycarbonyl) neomycin B (30). A stirred solution of neomycin B sulfate salt (5 g, 7.02 mmol) in water (200 mL) was treated with sodium carbonate (4.5 g, 42.12 mmol) at RT and cooled to 0 °C before a solution of *N*-(benzylloxycarbonyloxy)-succinimide (1.8 g, 7.22 mmol) in dioxane (200 mL) was added dropwise over a period of 6 h. The reaction was slowly allowed to warm to RT and stirring was continued for 18 h. Then the reaction was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH/NH₄OH (2:2:1) to give an inseparable 1:1 mixture of **28** and **29** (1.7 g, 32%, ESIHRMS calculated for C₃₁H₅₃N₆O₁₅ [M+H]⁺, 749.3569; found, 749.3555.). This regioisomeric mixture was used for next reaction without further purification. Along with the mono-Cbz compounds the di-Cbz compound **30** was also obtained (1.8 g, 29%). [α]^{RT}_D +69.6 (*c* .25, MeOH). ESIHRMS calculated for C₃₉H₅₉N₆O₁₇ [M+H]⁺, 883.3937; found, 883.3917.

6'-N-Benzylloxycarbonyl-1,3,2',2''',6'''-pentaazido-1,3,2',2''',6'''-pentadeamino neomycin B (31) and 6'''-N-Benzylloxycarbonyl-1,3,2',6',2''', -pentaazido-1,3,2',6',2''',-pentadeamino neomycin B (32). A stirred solution of 1:1 mixture of **28** and **29** (4.2 g, 5.61 mmol) in a mixture of methanol and water (2:3, 100 mL) was treated with potassium carbonate (6.2 g, 44.9 mmol) at RT and cooled to 0 °C before imidazole-1-sulfonyl azide hydrochloride (7.0 g, 33.67 mmol) and copper (II) sulfate (90 mg, 0.56 mmol) were added. The reaction was slowly allowed to warm to RT and stirring was continued for 18 h. Then the reaction was concentrated roughly to half of the initial volume under reduced pressure and extracted with ethyl acetate/THF (1:1, 3 x 50 mL). The combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH (9:1) to give an inseparable 1:1 mixture of **31** and **32** (2.6 g, 53%). This regioisomeric mixture was used

for next reaction without further purification. ESIHRMS calculated for $C_{31}H_{42}N_{16}O_{15}Na$ $[M+Na]^+$, 901.2896; found, 749.3555.

1,3,2',2'',6'''-Pentaazido-1,3,2',2'',6'''-pentadeamino neomycin B (33) and 1,3,2',6',2'''-Pentaazido-1,3,2',6',2'''-pentadeamino neomycin B (34). To a stirred solution of 1:1 mixture of **31** and **32** (2.5 g, 2.86 mmol) in dioxane (30 mL) was added 2M NaOH (30 mL). After stirring for 18 h at 60 °C the reaction was concentrated to dryness, dissolved in methanol (150 mL), and the precipitate was filtered off. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3/MeOH/NH_4OH$ (14:5:1) to give a 1:1 mixture of **33** and **34** (1.2 g, 57%). Careful separation of the mixture of **33** and **34** on silica gel eluting with $CHCl_3/MeOH/NH_4OH$ (14:5:1) gave the pure samples of regioisomers **33** and **34**.

1,3,2',2'',6'''-Pentaazido-1,3,2',2'',6'''-pentadeamino neomycin B (33). $[\alpha]_D^{RT} +89.7$ (c 1.09, MeOH). 1H NMR (600 MHz, CD_3OD) δ 5.83 (d, $J = 3.6$ Hz, 1H), 5.38 (br s, 1H), 5.11 (d, $J = 1.2$ Hz, 1H), 4.43 (dd, $J_1 = 6.7$ Hz, $J_2 = 4.7$ Hz, 1H), 4.28 (d, $J = 3.1$ Hz, 1H), 4.10 – 4.16 (m, 1H), 4.04 – 3.98 (m, 1H), 3.94 – 3.86 (m, 3H), 3.82 (dd, $J_1 = 11.9$ Hz, $J_2 = 2.4$ Hz, 1H), 3.74 – 3.61 (m, 5H), 3.57 – 3.49 (m, 1H), 3.47 (t, $J = 9.3$ Hz, 1H), 3.44 – 3.40 (m, 2H), 3.37 (dd, $J_1 = 12.9$ Hz, $J_2 = 4.3$ Hz, 1H), 3.24 (t, $J = 9.4$ Hz, 1H), 3.08 (dd, $J_1 = 10.5$ Hz, $J_2 = 3.6$ Hz, 1H), 3.04 (d, $J = 13.4$ Hz, 1H), 2.78 (dd, $J_1 = 13.4$ Hz, $J_2 = 6.7$ Hz, 1H), 2.17 (dt, $J_1 = 12.5$ Hz, $J_2 = 4.1$ Hz, 1H), 1.40 (q, $J = 12.5$ Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 107.7, 98.3, 96.4, 84.0, 81.9, 75.7, 75.6, 74.9, 74.2, 73.6, 72.0, 72.0, 70.7, 69.7, 68.1, 63.2, 61.9, 60.4, 60.3, 60.2, 51.0, 42.1, 31.6. ESIHRMS calculated for $C_{23}H_{37}N_{16}O_{13}$ $[M+H]^+$, 745.2726; found, 745.2722.

1,3,2',6',2'''-Pentaazido-1,3,2',6',2'''-,pentadeamino neomycin B (34). [α]^{RT}_D +101.8 (*c* 1.0, MeOH). ¹H NMR (600 MHz, CD₃OD) δ 5.76 (d, *J* = 3.7 Hz, 1H), 5.37 (br s, 1H), 5.11 (br s, 1H), 4.52 (dd, *J*₁ = 6.7 Hz, *J*₂ = 4.7 Hz, 1H), 4.32 (d, *J* = 4.2 Hz, 1H), 4.19 – 4.13 (m, 1H), 4.13 – 4.08 (m, 1H), 3.98 (d, *J* = 6.8 Hz, 1H), 3.93 (m, 1H), 3.85 (t, *J* = 9.6 Hz, 1H), 3.82 (dd, *J*₁ = 12.0 Hz, *J*₂ = 3.3 Hz, 1H), 3.74 (dd, *J*₁ = 12.0 Hz, *J*₂ = 3.9 Hz, 1H), 3.70 – 3.60 (m, 3H), 3.56 – 3.46 (m, 3H), 3.46 – 3.39 (m, 3H), 3.34 (t, *J* = 9.4 Hz, 1H), 3.27 – 3.13 (m, 1H), 3.11 (dd, *J*₁ = 10.5 Hz, *J*₂ = 3.7 Hz, 1H), 3.05 – 2.93 (m, 1H), 2.21 (dt, *J*₁ = 12.6 Hz, *J*₂ = 3.9 Hz, 1H), 1.37 (q, *J* = 12.6 Hz, 1H). ¹³C NMR (150 MHz, CD₃OD) δ 108.1, 98.4, 97.0, 83.5, 81.4, 76.1, 75.7, 75.2, 73.5, 73.36, 71.7, 71.2, 70.7, 69.9, 68.7, 63.2, 60.9, 60.5, 60.2, 59.7, 51.2, 41.2, 31.6. ESIHRMS calculated for C₂₃H₃₇N₁₆O₁₃ [M+H]⁺, 745.2726; found, 745.2704.

6'-N-(2-Benzyloxyethyl)-1,3,2',2''',6'''-pentaazido-1,3,2',2''',6'''-pentadeamino neomycin B (35) and 6'''-N-(2-Benzyloxyethyl)-1,3,2',6',2''', -pentaazido-1,3,2',6',2''',-pentadeamino neomycin B (36). To a stirred solution of 1:1 mixture of **33** and **34** (400 mg, 0.54 mmol) in methanol (9 mL) was added a solution of benzyloxyacetaldehyde (97 mg, 0.64 mmol) in methanol (1 mL) at RT. After stirring for 0.5 h at RT to the reaction was added glacial acetic acid (62 μ L, 1.07 mmol) and sodium cyanoborohydride (68 mg, 1.07 mmol) and stirring was continued for 5 h. The reaction was quenched with aq. NaHCO₃ (5 mL) followed by addition of DABCO (121 mg, 1.07 mmol) at RT. After stirring for 0.5 h the reaction was concentrated to dryness, dissolved in methanol (20 mL), and the precipitate was filtered off. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH (3:1) to give a 1:1 mixture of **35** and **36** (220 mg, 47%). The mixture of **35** and **36** was separated by preparative reversed-phase HPLC [column: Varian Microsorb 100-5 C18, Dynamax 21.4 x 250 mm, 5.0 micron, protected by a Varian Dynamax HPLC guard column (21.4

mm, compression module, Microsorb Guard-8 C18); temperature: ambient; flow rate: 21.5 mL min⁻¹; mobile phase: (A) water, (B) acetonitrile; gradient program: 0-30 min, 22.5% B, detector wavelength: 220 nm] to give pure samples of **35** and **36**.

6'-N-(2-Benzoyloxyethyl)-1,3,2',2''',6'''-pentaazido-1,3,2',2''',6'''-pentadeamino neomycin B (35). [α]_D^{RT} +89.6 (*c* 0.87, MeOH). ¹H NMR (600 MHz, CD₃OD) δ 7.34 (m, 5H), 5.93 (d, *J* = 3.3 Hz, 1H), 5.35 (br s, 1H), 5.11 (br s, 1H), 4.59 (br s, 2H), 4.37 (t, *J* = 5.5 Hz, 1H), 4.31 (d, *J*_I = 3.9 Hz, 1H), 4.21 (t, *J* = 8.5 Hz, 1H), 4.11 (td, *J*_I = 6.0 Hz, *J*₂ = 2.2 Hz, 1H), 4.02 – 3.97 (m, 1H), 3.94 – 3.91 (m, 1H), 3.86 (t, *J* = 9.6 Hz, 1H), 3.82 (dd, *J*_I = 11.8 Hz, *J*₂ = 1.4 Hz, 1H), 3.77 – 3.54 (m, 8H), 3.49 – 3.28 (m, 8H), 3.24 (t, *J* = 9.4 Hz, 1H), 3.15 (dd, *J*_I = 6.0 Hz, *J*₂ = 2.2 Hz, 1H), 2.19 (dt, *J*_I = 12.6 Hz, *J*₂ = 4.1 Hz, 1H), 1.28 (q, *J* = 12.5 Hz, 1H). ¹³C NMR (150 MHz, CD₃OD) δ 137.3, 128.2, 127.8, 127.7, 109.0, 98.4, 96.7, 84.0, 82.1, 76.1, 75.9, 75.7, 74.2, 73.7, 73.1, 72.2, 70.5, 69.7, 68.1, 67.5, 64.4, 62.9, 62.3, 60.4, 60.3, 60.1, 51.1, 48.8, 47.2, 31.7. ESIHRMS calculated for C₃₂H₄₇N₁₆O₁₄ [M+H]⁺, 878.3458; found, 878.3412.

6'''-N-(2-Benzoyloxyethyl)-1,3,2',6',2''', -pentaazido-1,3,2',6',2''',-pentadeamino neomycin B (36). [α]_D^{RT} +71.3 (*c* 1.5, MeOH). ¹H NMR (600 MHz, CD₃OD) δ 7.42 – 7.24 (m, 5H), 5.74 (d, *J* = 3.7 Hz, 1H), 5.34 (d, *J* = 1.2 Hz, 1H), 5.15 (d, *J* = 1.4 Hz, 1H), 4.65 – 4.56 (m, 2H), 4.1 (dd, *J*_I = 4.5 Hz, *J*₂ = 6.8 Hz, 1H), 4.30 (d, *J* = 4.5 Hz, 1H), 4.25 – 4.19 (m, 1H), 4.18 – 4.13 (m, 1H), 4.08 (dt, *J*_I = 6.9 Hz, *J*₂ = 3.4 Hz, 1H), 3.96 (t, *J* = 3.2 Hz, 1H), 3.85 (t, *J* = 10.3 Hz, 1H), 3.79 (dd, *J*_I = 12.2 Hz, *J*₂ = 3.5 Hz, 1H), 3.77 – 3.70 (m, 3H), 3.68 – 3.57 (m, 3H), 3.57 – 3.48 (m, 3H), 3.47 (dd, *J*_I = 13.3 Hz, *J*₂ = 8.1 Hz, 1H), 3.42 (dd, *J*_I = 13.2 Hz, *J*₂ = 5.5 Hz, 1H), 3.41 – 3.37 (m, 2H), 3.36 – 3.26 (m, 4H), 3.10 (dd, *J*_I = 10.5 Hz, *J*₂ = 3.8 Hz, 1H), 2.20 (dt, *J*_I = 12.5 Hz, *J*₂ = 4.2 Hz, 1H), 1.34 (q, *J* = 12.5 Hz, 1H). ¹³C NMR (150 MHz, CD₃OD) δ 137.4, 128.2, 127.8, 127.7, 108.1, 98.5, 97.1, 83.6, 81.1, 76.2, 75.7, 75.4, 73.5, 72.9, 71.7, 71.2, 70.6, 70.0, 69.6,

68.7, 64.1, 63.2, 60.5, 60.5, 59.7, 59.7, 51.2, 48.5, 47.4, 31.5. ESIHRMS calculated for $C_{32}H_{46}N_{16}O_{14}Na [M+Na]^+$, 901.3277; found, 901.3257.

6'-N-(2-Hydroxyethyl) neomycin B acetate salt (37). To a stirred solution of **35** (35 mg, 0.04 mmol) in a mixture of THF and water (1:1, 1.5 mL) was added 1M NaOH (0.5 mL) and trimethylphosphine (1M in THF, 0.26 mL). After stirring for 3 h at 60 °C the reaction was concentrated to dryness under reduced pressure. The residue was roughly purified by column chromatography on silica gel eluting with $CHCl_3/MeOH/NH_4OH$ (2:2:1). The product-containing fractions were concentrated under reduced pressure to give an off-white solid (20 mg). The solid was dissolved in methanol (1 mL) and added to a stirred suspension of $Pd(OH)_2/C$ (20 mg) in 10% AcOH (1 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 4 h, filtered, concentrated and purified by Sephadex C-25 column chromatography (1.5% ammonium hydroxide). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to give the desired product **37** as the peracetate salt (10 mg, 23 %). $[\alpha]_D^{RT} +27.8$ (c 0.14, H_2O). 1H NMR (600 MHz, D_2O) δ 5.85 (d, $J = 3.9$ Hz, 1H), 5.22 (d, $J = 2.3$ Hz, 1H), 5.08 (d, $J = 1.4$ Hz, 1H), 4.29 (dd, $J_1 = 6.3$ Hz, $J_2 = 5.1$ Hz, 1H), 4.20 (dd, $J_1 = 4.8$ Hz, $J_2 = 2.4$ Hz, 1H), 4.12 – 4.07 (m, 1H), 4.12 – 4.07 (m, 2H), 3.89 (t, $J = 9.7$ Hz, 1H), 3.83 – 3.76 (m, 2H), 3.73 (t, $J = 9.2$ Hz, 1H), 3.70 (dd, $J_1 = 12.4$ Hz, $J_2 = 2.7$ Hz, 1H), 3.68 – 3.63 (m, 2H), 3.63 – 3.59 (m, 1H), 3.54 (dd, $J_1 = 12.3$ Hz, $J_2 = 5.0$ Hz, 1H), 3.58 (t, $J = 9.8$ Hz, 1H), 3.39 – 3.36 (m, 1H), 3.34 (dd, $J_1 = 13.2$ Hz, $J_2 = 3.2$ Hz, 1H), 3.33 – 3.28 (m, 1H), 3.26 (t, $J = 9.5$ Hz, 1H), 3.25 – 3.18 (m, 2H), 3.18 – 3.11 (m, 3H), 3.08 – 3.02 (m, 2H), 2.27 (dt, $J_1 = 12.6$ Hz, $J_2 = 4.0$ Hz, 1H), 1.81 (s, 18H), 1.67 (q, $J = 12.6$ Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 178.1, 110.1, 95.3, 95.3, 84.7, 81.5, 75.1, 75.1, 73.5, 72.4, 70.9, 70.0, 69.2, 67.8,

67.5, 67.2, 60.0, 56.1, 53.3, 50.7, 49.9, 49.7, 48.2, 48.2, 40.3, 28.0, 21.2. ESIHRMS calculated for $C_{25}H_{50}N_6O_{14}Na$ $[M+Na]^+$, 681.3283; found, 681.3286.

6'''-N-(2-Hydroxyethyl) neomycin B acetate salt (38). To a stirred solution of **36** (75 mg, 0.08 mmol) in a mixture of THF and water (1:1, 3 mL) was added 1M NaOH (1 mL) and trimethylphosphine (1M in THF, 0.6 mL). After stirring for 3 h at 60 °C the reaction was concentrated to dryness under reduced pressure. The residue was roughly purified by column chromatography on silica gel eluting with $CHCl_3/MeOH/NH_4OH$ (2:2:1). The product-containing fractions were concentrated under reduced pressure to give an off-white solid (50 mg). The solid was dissolved in methanol (2 mL) and added to a stirred suspension of $Pd(OH)_2/C$ (50 mg) in 10 % AcOH (2 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 4 h, filtered, concentrated and purified by Sephadex C-25 column chromatography (1.5% ammonium hydroxide). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to give the desired product **38** as the peracetate salt (25 mg, 26 %). $[\alpha]_D^{RT} +39.0$ (c 0.5, H_2O). 1H NMR (600 MHz, D_2O) δ 5.83 (d, $J = 4.0$ Hz, 1H), 5.22 (d, $J = 2.3$ Hz, 1H), 5.09 (d, $J = 1.3$ Hz, 1H), 4.29 (t, $J = 5.8$ Hz, 1H), 4.22 – 4.16 (m, 2H), 4.05 – 3.99 (m, 2H), 3.87 (t, $J = 9.7$ Hz, 1H), 3.79 (dd, $J_1 = 10.7$ Hz, $J_2 = 9.2$ Hz, 1H), 3.77 – 3.68 (m, 3H), 3.66 (t, $J = 5.2$ Hz, 2H), 3.63 – 3.60 (m, 1H), 3.53 (dd, $J_1 = 12.3$ Hz, $J_2 = 5.0$ Hz, 1H), 3.48 (t, $J = 9.8$ Hz, 1H), 3.41 – 3.27 (m, 3H), 3.27 – 3.20 (m, 4H), 3.14 (dt, $J_1 = 10.9$ Hz, $J_2 = 3.5$ Hz, 1H), 3.10 – 3.02 (m, 3H), 2.27 (dt, $J_1 = 12.6$ Hz, $J_2 = 4.1$ Hz, 1H), 1.83 (s, 18H), 1.68 (q, $J = 12.6$ Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 178.9, 110.1, 95.3, 84.7, 81.4, 75.2, 75.1, 73.4, 72.4, 70.6, 69.7, 69.3, 67.9, 67.5, 67.1, 60.00, 56.3, 53.4, 50.7, 49.7, 49.6, 48.8, 48.3, 47.9, 40.1, 28.1, 21.8. ESIHRMS calculated for $C_{25}H_{50}N_6O_{14}Na$ $[M+Na]^+$, 681.3283; found, 681.3262.

6',6'''-Bis-*N*-(benzyloxycarbonyl)-1,3,2',2'''-tetraazido-1,3,2',2'''-tetradeamino neomycin B (39). A stirred solution of **30** (1.7 g, 1.93 mmol) in a mixture of methanol and water (1:1, 50 mL) was treated with potassium carbonate (2.13 g, 15.41 mmol) at RT and cooled to 0 °C before imidazole-1-sulfonyl azide hydrochloride (2.0 g, 9.63 mmol) and copper (II) sulfate (31 mg, 0.19 mmol) were added. The reaction was slowly allowed to warm to RT and stirring was continued for 18 h. Then the reaction was concentrated roughly to half of the initial volume under reduced pressure and extracted with ethyl acetate/THF (1:1, 3 x 50 mL). The combined organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The residue was roughly purified by column chromatography on silica gel eluting with CHCl₃/MeOH (9:1) to give the desired product **39** (500 mg, 26%). [α]_D^{RT} +72.0 (*c* 1.33, MeOH). ESIHRMS calculated for C₃₉H₅₀N₁₄O₁₇Na [M+Na]⁺, 1009.3376; found, 1009.3319.

1,3,2',2'''-Tetraazido-1,3,2',2'''-tetradeamino neomycin B (40). To a stirred solution of **39** (450 mg, 0.46 mmol) in dioxane (10 mL) was added 4M NaOH (10 mL). After stirring for 18 h at 60 °C the reaction was concentrated to dryness, dissolved in methanol (20 mL), and the precipitate was filtered off. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH/NH₄OH (3:2:1) to give the desired product **24** (190 mg, 58%). [α]_D^{RT} +123.9 (*c* 0.67, MeOH). ¹H NMR (600 MHz, CD₃OD) δ 5.76 (d, *J* = 3.4 Hz, 1H), 5.38 (br s, 1H), 5.09 (br s, 1H), 4.54 (dd, *J*₁ = 6.8 Hz, *J*₂ = 4.6 Hz, 1H), 4.28 (d, *J* = 4.2 Hz, 1H), 4.14 – 4.08 (m, 1H), 3.95 – 3.85 (m, 3H), 3.81 (dd, *J*₁ = 11.9 Hz, *J*₂ = 2.7 Hz, 1H), 3.77 – 3.71 (m, 2H), 3.66 (t, *J* = 9.3 Hz, 1H), 3.62 (br s, 1H), 3.56 – 3.50 (m, 1H), 3.50 (t, *J* = 9.5 Hz, 1H), 3.46 (br s, 1H), 3.44 – 3.37 (m, 1H), 3.25 (t, *J* = 9.2 Hz, 1H), 3.10 (dd, *J*₁ = 10.4 Hz, *J*₂ = 3.6 Hz, 1H), 3.15 – 2.98 (m, 2H), 2.93 – 2.72 (m, 2H), 2.17 (dt, *J*₁ = 12.7 Hz, *J*₂ = 4.3 Hz, 1H), 1.40 (q, *J* = 12.6 Hz, 1H). ¹³C NMR (150 MHz, CD₃OD) δ 106.9, 98.4, 96.4,

83.5, 81.6, 75.2, 75.1, 74.8, 73.4, 72.2, 72.0, 70.7, 70.0, 68.9, 63.3, 60.6, 60.4, 60.3, 60.2, 42.0, 41.6, 31.6. ESIHRMS calculated for $C_{23}H_{39}N_{14}O_{13}$ $[M+H]^+$, 719.2821; found, 719.2794.

6',6'''-Bis-N-(2-hydroxyethyl) neomycin B acetate salt (42). To a stirred solution of **24** (190 mg, 0.26 mmol) in methanol (3 mL) was added a solution of benzyloxyacetaldehyde (92 mg, 0.61 mmol) in methanol (0.5 mL) at RT. After stirring for 0.5 h at RT to the reaction was added glacial acetic acid (60 μ L, 1.06 mmol) and sodium cyanoborohydride (67 mg, 1.06 mmol) and stirring was continued for 4 h. The reaction was quenched with aq. $NaHCO_3$ (2 mL) followed by addition of DABCO (120 mg, 1.06 mmol) at RT. After stirring for 0.5 h the reaction was concentrated to dryness, dissolved in methanol (10 mL), and the precipitate was filtered off. The filtrate was concentrated under reduced pressure. The residue was roughly purified by column chromatography on silica gel eluting with $CHCl_3/MeOH$ (4:1) to give the desired product **41** (150 mg, 57%). The compound used for next reaction without further purification. ESIHRMS calculated for $C_{41}H_{59}N_{14}O_{15}Na$ $[M+Na]^+$, 987.4284; found, 987.4246.

To a stirred solution of **41** (150 mg, 0.15 mmol) in a mixture of THF and water (1:1, 4 mL) was added 1M NaOH (2 mL) and trimethylphosphine (1M in THF, 0.8 mL). After stirring for 3 h at 60 $^{\circ}C$ the reaction was concentrated to dryness under reduced pressure. The residue was roughly purified by column chromatography on silica gel eluting with $CHCl_3/MeOH/NH_4OH$ (2:2:1). The product-containing fractions were concentrated under reduced pressure to give an off-white solid (100 mg). The solid was dissolved in methanol (3 mL) and added to a stirred suspension of $Pd(OH)_2/C$ (100 mg) in 10% AcOH (3 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 12 h, filtered, concentrated and purified by Sephadex C-25 column chromatography (0.6% ammonium hydroxide). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to

give the desired product **42** as the peracetate salt (25 mg, 15%). $[\alpha]_{\text{D}}^{\text{RT}} +63.3$ (c 0.67, H₂O). ¹H NMR (600 MHz, D₂O) δ 5.84 (d, J = 3.8 Hz, 1H), 5.22 (d, J = 2.1 Hz, 1H), 5.08 (br s, 1H), 4.28 (t, J = 5.5 Hz, 1H), 4.21 – 4.16 (m, 2H), 4.05 – 3.98 (m, 2H), 3.89 (t, J = 9.7 Hz, 1H), 3.83 – 3.77 (m, 2H), 3.72 (t, J = 9.1 Hz, 1H), 3.70 (dd, J_1 = 11.5 Hz, J_2 = 2.4 Hz, 1H), 3.65 (t, J = 5.0 Hz, 4H), 3.60 (br s, 1H), 3.52 (dd, J_1 = 12.5 Hz, J_2 = 4.9 Hz, 1H), 3.47 (t, J = 9.7 Hz, 1H), 3.38 (br s, 1H), 3.34 (dd, J_1 = 13.4 Hz, J_2 = 4.8 Hz, 1H), 3.30 (dd, J_1 = 10.5 Hz, J_2 = 3.7 Hz, 1H), 3.28 – 3.20 (m, 4H), 3.18 – 3.10 (m, 2H), 3.09 – 3.02 (m, 4H), 2.27 (dt, J_1 = 12.5 Hz, J_2 = 3.8 Hz, 1H), 1.80 (s, 18 H), 1.67 (q, J = 12.6 Hz, 1H). ¹³C NMR (150 MHz, D₂O) δ 178.1, 110.1, 95.3, 95.2, 84.8, 81.4, 75.1, 73.5, 72.4, 70.9, 69.7, 69.2, 67.8, 67.4, 67.1, 60.0, 56.3, 56.2, 53.4, 50.7, 49.9, 49.7, 49.6, 48.8, 48.3, 48.2, 47.9, 28.0, 21.3. ESIHRMS calculated for C₂₇H₅₄N₆O₁₅Na [M+Na]⁺, 725.3545; found, 725.3514.

6'-N-Acetyl-1,3,2',2''',6'''-pentaazido-1,3,2',2''',6'''-pentadeamino neomycin B (43). To a stirred solution of **33** (90 mg, 0.12 mmol) in MeOH (1 mL) was added acetic anhydride (1 mL) at RT. After stirring for 24 h at RT the reaction was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH (9:1) to give the desired product **43** (60 mg, 63%). $[\alpha]_{\text{D}}^{\text{RT}} +95.2$ (c 0.9, MeOH). ¹H NMR (600 MHz, CD₃OD) δ 5.74 (d, J = 3.8 Hz, 1H), 5.36 (d, J = 6.5 Hz, 1H), 5.11 (d, J = 1.2 Hz, 1H), 4.42 (dd, J_1 = 6.5 Hz, J_2 = 4.7 Hz, 1H), 4.29 (d, J = 3.3 Hz, 1H), 4.13 (m, 1H), 4.03 – 3.99 (m, 1H), 3.98 – 3.94 (m, 1H), 3.93 (t, J = 3.3 Hz, 1H), 3.86 (t, J = 9.6 Hz, 1H), 3.82 (dd, J_1 = 11.8 Hz, J_2 = 2.4 Hz, 1H), 3.72 – 3.60 (m, 5H), 3.57 (dd, J_1 = 11.2 Hz, J_2 = 4.8 Hz, 1H), 3.55 – 3.47 (m, 2H), 3.47 – 3.40 (m, 2H), 3.38 (dd, J_1 = 13.0 Hz, J_2 = 4.4 Hz, 1H), 3.34 – 3.28 (m, 1H), 3.17 (t, J = 9.4 Hz, 1H), 3.08 (dd, J_1 = 10.5 Hz, J_2 = 3.8 Hz, 1H), 2.19 (dt, J_1 = 12.2 Hz, J_2 = 3.9 Hz, 1H), 1.96 (s, 2H), 1.33 (q, J = 12.3 Hz, 1H). ¹³C NMR (150 MHz, CD₃OD) δ 172.5, 107.9, 98.3,

96.8, 83.8, 82.0, 75.8, 75.7, 75.5, 74.2, 73.7, 72.4, 71.8, 71.0, 70.5, 69.7, 68.2, 63.2, 63.0, 62.1, 60.4, 60.3, 59.9, 51.1, 40.0, 31.7, 21.2. ESIHRMS calculated for $C_{25}H_{38}N_{16}O_{14}Na$ $[M+Na]^+$, 809.2651; found, 809.2642.

6'-N-Acetyl neomycin B acetate salt (44). To a stirred suspension of Pd/C (110 mg) in 10% AcOH (1 mL) was added a solution of **43** (55 mg, 0.07 mmol) in dioxane (2.0 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 18 h, filtered, concentrated and purified by Sephadex C-25 column chromatography (0.6% ammonium hydroxide). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to give the desired product **44** as the peracetate salt (30 mg, 44%). $[\alpha]^{RT}_D +45.0$ (*c* 0.4, H_2O). 1H NMR (600 MHz, D_2O) δ 5.58 (d, J = 4.0 Hz, 1H), 5.12 (d, J = 2.0 Hz, 1H), 5.00 (d, J = 1.5 Hz, 1H), 4.24 (dd, J_1 = 6.7 Hz, J_2 = 4.9 Hz, 1H), 4.13 (dd, J_1 = 4.7 Hz, J_2 = 2.2 Hz, 1H), 4.05 – 4.01 (m, 1H), 3.97 – 3.91 (m, 2H), 3.75 (t, J = 9.6 Hz, 1H), 3.69 – 3.58 (m, 3H), 3.57 – 3.44 (m, 3H), 3.41 (t, J = 9.9 Hz, 1H), 3.36 – 3.28 (m, 2H), 3.28 – 3.19 (m, 2H), 3.19 – 3.02 (m, 5H), 2.22 (dt, J_1 = 12.6 Hz, J_2 = 4.0 Hz, 1H), 1.74 (s, 18H), 1.59 (q, J = 12.6 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 178.1, 178.0, 174.5, 109.9, 95.3, 95.1, 84.3, 81.2, 75.8, 75.1, 73.3, 72.2, 71.8, 70.0, 70.0, 68.3, 67.4, 67.1, 60.0, 53.5, 50.7, 49.6, 48.5, 40.2, 39.2, 27.9, 21.7, 21.3. ESIHRMS calculated for $C_{25}H_{49}N_6O_{14}$ $[M+H]^+$, 657.3307; found, 657.3300.

1,3,2',6',2'''-Pentaazido-6'''-hydroxy-1,3,2',6',2'''',6'''-hexadeamino neomycin B (45). A stirred solution of **34** (140 mg, 0.19 mmol) in 5% AcOH (3 mL) was treated with sodium nitrite (65 mg, 0.94 mmol) at 0 °C. After stirring for 2 h at 0 °C the reaction was quenched with aq. $NaHCO_3$ (5 mL). Then the reaction was concentrated to dryness, dissolved in methanol (20 mL), and the precipitate was filtered off. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3/MeOH$ (6:1) to

give the desired product **45** (55 mg, 39%). $[\alpha]_{\text{D}}^{\text{RT}} +50.4$ (*c* 1.0, MeOH). ^1H NMR (600 MHz, CD_3OD) δ 5.76 (d, $J = 3.8$ Hz, 1H), 5.35 (d, $J = 1.1$ Hz, 1H), 5.08 (d, $J = 1.7$ Hz, 1H), 4.49 (dd, $J_1 = 7.0$ Hz, $J_2 = 4.6$ Hz, 1H), 4.32 (dd, $J_1 = 4.4$ Hz, $J_2 = 1.1$ Hz, 1H), 4.15 (ddd, $J_1 = 9.7$ Hz, $J_2 = 5.7$ Hz, $J_3 = 2.2$ Hz, 1H), 4.13 – 4.09 (m, 1H), 3.94 – 3.89 (m, 2H), 3.88 – 3.79 (m, 3H), 3.74 (dd, $J_1 = 7.4$ Hz, $J_2 = 4.6$ Hz, 1H), 3.72 (dd, $J_1 = 7.4$ Hz, $J_2 = 6.8$ Hz, 1H), 3.66 – 3.60 (m, 3H), 3.54 – 3.47 (m, 3H), 3.42 (m, 3H), 3.33 (t, $J = 9.4$ Hz, 1H), 3.12 (dd, $J_1 = 10.5$ Hz, $J_2 = 3.8$ Hz, 1H), 2.21 (dt, $J_1 = 12.5$ Hz, $J_2 = 4.2$ Hz, 1H), 1.37 (q, $J = 12.5$ Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 108.0, 98.4, 97.0, 83.5, 81.8, 76.1, 75.7, 75.6, 75.4, 73.5, 71.7, 71.2, 70.8, 69.8, 68.3, 63.2, 61.5, 61.4, 60.6, 60.4, 59.7, 51.2, 31.6. ESIHRMS calculated for $\text{C}_{23}\text{H}_{35}\text{N}_{15}\text{O}_{14}\text{Na}$ $[\text{M}+\text{Na}]^+$, 768.2386; found, 768.2352.

6'''-Deamino-6'''-hydroxy neomycin B acetate salt (46). A stirred solution of **45** (55 mg, 0.15 mmol) in a mixture of THF and water (1:1, 2 mL) was added 1M NaOH (1 mL) and trimethylphosphine (1M in THF, 0.5 mL). After stirring for 3 h at 60 $^{\circ}\text{C}$ the reaction was concentrated to dryness under reduced pressure. The residue was purified by Sephadex C-25 column chromatography (1.0 % ammonium hydroxide). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to give the desired product **46** as the peracetate salt (25 mg, 35%). $[\alpha]_{\text{D}}^{\text{RT}} +42.9$ (*c* 0.68, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.80 (d, $J = 2.9$ Hz, 1H), 5.19 (br s, 1H), 5.00 (br s, 1H), 4.29 – 4.27 (t, $J = 5.3$ Hz, 1H), 4.21 – 4.18 (m, 1H), 4.03 – 3.96 (m, 2H), 3.87 – 3.83 (m, 1H), 3.83 (t, $J = 9.8$ Hz, 1H), 3.77 (t, $J = 10.0$ Hz, 1H), 3.74 – 3.67 (m, 3H), 3.67 – 3.56 (m, 3H), 3.54 (d, $J = 1.3$ Hz, 1H), 3.46 (t, $J = 9.8$ Hz, 1H), 3.32 (d, $J = 1.3$ Hz, 1H), 3.32 – 3.18 (m, 4H), 3.12 (ddd, $J_1 = 10.9$ Hz, $J_2 = 8.7$ Hz, $J_3 = 3.8$ Hz, 1H), 3.04 (dd $J_1 = 13.1$ Hz, $J_2 = 6.3$ Hz, 1H), 2.25 (dt, $J_1 = 12.8$ Hz, $J_2 = 3.9$ Hz, 1H), 1.75 (s, 15H), 1.65 (q, $J = 12.8$ Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 179.5, 110.0, 95.4,

95.4, 84.6, 81.4, 75.4, 75.1, 74.9, 73.6, 72.4, 70.5, 69.3, 67.9, 67.6, 66.4, 61.1, 59.9, 53.5, 51.1, 49.7, 48.3, 40.0, 28.1, 22.1. ESIHRMS calculated for $C_{23}H_{46}N_5O_{14}$ $[M+H]^+$, 616.3041; found, 616.3030.

1,3,2',2''',6'''-Pentazido-4'-O-(ethyl)-1,3,2',2''',6'''-pentadeamino paromomycin (47).

A stirred solution of **22** (500 mg, 0.53 mmol) in a mixture of methanol and water (1:2, 15 mL) was treated with potassium carbonate (732 mg, 5.30 mmol) at RT and cooled to 0 °C before imidazole-1-sulfonyl azide hydrochloride (667 mg, 3.18 mmol) and copper (II) sulfate (8.5 mg, 0.05 mmol) were added. The reaction was slowly allowed to warm to RT and stirring was continued for 18 h. Then the reaction was concentrated to dryness, dissolved in methanol (50 mL), and the precipitate was filtered off. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3/MeOH$ (9:1) to give the desired product **47** (235 mg, 57%). $[\alpha]_D^{RT} +97.8$ (c 1.0, MeOH). 1H NMR (600 MHz, CD_3OD) δ 5.77 (d, $J = 3.6$ Hz, 1H), 5.36 (d, $J = 1.2$ Hz, 1H), 5.12 (d, $J = 1.3$ Hz, 1H), 4.44 (dd, $J_1 = 6.4$ Hz, $J_2 = 4.8$ Hz, 1H), 4.28 (dd, $J_1 = 4.4$ Hz, $J_2 = 1.4$ Hz, 1H), 4.16 – 4.10 (m, 1H), 4.03 – 3.97 (m, 2H), 3.93 (t, $J = 3.3$ Hz, 1H), 3.92 – 3.86 (m, 2H), 3.82 (dd, $J_1 = 12.0$ Hz, $J_2 = 2.5$ Hz, 1H), 3.79 (dd, $J_1 = 11.9$ Hz, $J_2 = 2.1$ Hz, 1H), 3.74 – 3.64 (m, 6H), 3.63 (dd, $J_1 = 12.9$ Hz, $J_2 = 8.5$ Hz, 1H), 3.52 – 3.38 (m, 4H), 3.38 (dd, $J_1 = 13.0$ Hz, $J_2 = 4.6$ Hz, 1H), 3.24 (t, $J = 9.4$ Hz, 1H), 3.07 (dd, $J_1 = 10.6$ Hz, $J_2 = 3.7$ Hz, 1H), 2.16 (dt, $J_1 = 12.8$ Hz, $J_2 = 4.3$ Hz, 1H), 1.36 (q, $J = 12.7$ Hz, 1H), 1.18 (t, $J = 12.7$ Hz, 3H). ^{13}C NMR (151 MHz, CD_3OD) δ 107.5, 98.3, 96.5, 83.8, 82.0, 78.2, 75.7, 75.5, 75.0, 74.1, 73.7, 71.9, 70.9, 69.7, 68.1, 68.0, 63.3, 61.8, 60.5, 60.4, 60.4, 60.0, 51.0, 31.6, 14.6. ESIHRMS calculated for $C_{25}H_{39}N_{15}O_{14}Na$ $[M+Na]^+$, 796.2699; found, 796.2679.

1,3,2',2''',6'''-Pentazido-4'-O-ethyl-6'-O-(*p*-toluenesulfonyl)-1,3,2',2''',6'''-pentadeamino paromomycin (48), 1,3,2',2''',6'''-Pentazido-4'-O-ethyl-5''-O-(*p*-toluenesulfonyl)-1,3,2',2''',6'''-pentadeamino paromomycin (49), and 1,3,2',2''',6'''-Pentazido-4'-O-ethyl-6',5''-bis-O-(*p*-toluenesulfonyl)-1,3,2',2''',6'''-pentadeamino paromomycin (50). A stirred solution of **47** (150 mg, 0.19 mmol) in pyridine (1.5 mL) was treated with tosyl chloride (92 mg, 0.48 mmol) at RT. After stirring for 18 h at RT the reaction was quenched with methanol (10 mL) and concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH (12:1) to give an inseparable 1:0.7 mixture of **48** and **49** (55 mg, 30%, ESIHRMS calculated for C₃₂H₄₅N₁₅O₁₆NaS [M+Na]⁺, 950.2787; found, 950.2798.). This regioisomeric mixture was used for next reaction without further purification. Along with the mono-tosylated compounds the di-tosylated compound **50** was also obtained (21 mg, 10%).

1,3,2',2''',6'''-Pentazido-4'-O-ethyl-6',5''-bis-O-(*p*-toluenesulfonyl)-1,3,2',2''',6'''-pentadeamino paromomycin (50). [α]_D^{RT} +58.0 (*c* 0.25, MeOH). ¹H NMR (600 MHz, CD₃OD) δ 7.81 (d, *J* = 3.6 Hz, 2H), 7.79 (d, *J* = 3.6 Hz, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.42 (d, *J* = 8.0 Hz, 2H), 5.69 (d, *J* = 3.7 Hz, 1H), 5.28 (d, *J* = 1.9 Hz, 1H), 5.06 (d, *J* = 1.4 Hz, 1H), 4.32 – 4.25 (m, 4H), 4.24 – 4.19 (m, 2H), 4.19 (dd, *J*₁ = 10.7 Hz, *J*₂ = 1.7 Hz, 1H), 4.13 – 4.08 (m, 1H), 3.98 (ddd, *J*₁ = 6.6 Hz, *J*₂ = 4.6 Hz, *J*₃ = 1.6 Hz, 1H), 3.92 (t, *J* = 3.3 Hz, 1H), 3.91 – 3.83 (m, 2H), 3.65 (br s, 1H), 3.60 (dd, *J*₁ = 12.8 Hz, *J*₂ = 8.5 Hz, 1H), 3.53 (t, *J* = 8.9 Hz, 1H), 3.49 (dd, *J*₁ = 9.2 Hz, *J*₂ = 7.0 Hz, 1H), 3.45 – 3.40 (m, 2H), 3.39 (t, *J* = 5.0 Hz, 1H), 3.38 – 3.32 (m, 2H), 3.23 (t, *J* = 9.5 Hz, 1H), 3.13 (t, *J* = 9.5 Hz, 1H), 2.92 (dd, *J*₁ = 10.5 Hz, *J*₁ = 3.8 Hz, 1H), 2.45 (s, 3H), 2.43 (s, 3H), 2.14 (dt, *J*₁ = 12.5 Hz, *J*₂ = 4.5 Hz, 1H), 1.25 (q, *J* = 12.5 Hz, 1H), 1.06 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (150 MHz, CD₃OD) δ 145.2, 145.2, 132.8, 132.8, 129.9, 129.7, 127.8, 127.8, 110.0,

98.7, 96.2, 84.3, 79.2, 77.8, 76.4, 76.3, 75.7, 74.1, 73.4, 71.5, 70.2, 69.6, 69.1, 68.8, 68.1, 68.1, 63.4, 60.3, 60.2, 59.7, 50.9, 29.3, 20.4, 20.3, 14.6. ESIHRMS calculated for $C_{39}H_{59}N_6O_{17}$ $[M+H]^+$, 883.3937; found, 883.3917).

1,3,2',2''',6'''-Pentazido-4'-O-ethyl-6'-N-(2-hydroxyethyl)-1,3,2',2''',6'''-pentadeamino neomycin B (51), 1,3,2',2''',6'''-Pentazido-4'-O-ethyl-5''-deoxy-5''-(2-hydroxyethylamino)-1,3,2',2''',6'''-pentadeamino paromomycin (52). The 1:0.7 mixture of **48** and **49** (55 mg, 0.06 mmol) was dissolved in ethanolamine (0.5 mL) and the solution was stirred for 18 h at RT. Then the reaction was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $CHCl_3/MeOH$ (3:1) to give an inseparable 1:0.7 mixture of **51** and **52** (25 mg, 52 %). The mixture of **51** and **52** was separated by preparative reversed-phase HPLC [column: Varian Microsorb 100-5 C18, Dynamax 21.4 x 250 mm, 5.0 micron, protected by a Varian Dynamax HPLC guard column (21.4 mm, compression module, Microsorb Guard-8 C18); temperature: ambient; flow rate: 21.5 mL min^{-1} ; mobile phase: (A) water, (B) acetonitrile; gradient program: 0-30 min, 11% B, detector wavelength: 220 nm] to give reasonably pure samples of **35** (ESIHRMS calculated for $C_{27}H_{45}N_{16}O_{14}$ $[M+H]^+$, 817.3301; found, 817.3293) and **36** (ESIHRMS calculated for $C_{27}H_{45}N_{16}O_{14}$ $[M+H]^+$, 817.3301; found, 817.3296). These compounds were used for the next reactions without further purification and characterization.

4'-O-Ethyl-6'-N-(2-hydroxyethyl) neomycin B acetate salt (53). To a stirred solution of **51** (12 mg, 0.015 mmol) in a mixture of THF and water (1:1, 0.5 mL) was added 1M NaOH (0.25 mL) and trimethylphosphine (1M in THF, 150 μ L). After stirring for 2 h at 60 $^{\circ}$ C the reaction was concentrated to dryness under reduced pressure. The residue was purified by Sephadex C-25 column chromatography (0.8% ammonium hydroxide). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to

give the desired product **53** as the peracetate salt (6.5 mg, 42%). $[\alpha]_{\text{D}}^{\text{RT}} +46.4$ (*c* 0.22, H₂O). ¹H NMR (600 MHz, D₂O) δ 5.85 (d, *J* = 3.8 Hz, 1H), 5.23 (d, *J* = 2.4 Hz, 1H), 5.09 (d, *J* = 1.3 Hz, 1H), 4.29 (t, *J* = 5.7 Hz, 1H), 4.19 (dd, *J*₁ = 4.7 Hz, *J*₂ = 2.6 Hz, 1H), 4.11 (t, *J* = 4.9 Hz, 1H), 4.06 – 4.01 (m, 2H), 3.91 (t, *J* = 9.6 Hz, 1H), 3.89 (t, *J* = 9.7 Hz, 1H), 3.81 (td, *J*₁ = 9.0 Hz, *J*₂ = 2.6 Hz, 1H), 3.76 – 3.64 (m, 5H), 3.62 (d, *J* = 1.7 Hz, 1H), 3.58 – 3.51 (m, 2H), 3.49 (t, *J* = 9.8 Hz, 1H), 3.39 (d, *J* = 1.2 Hz, 1H), 3.35 – 3.30 (m, 2H), 3.28 (dd, *J*₁ = 10.6 Hz, *J*₂ = 3.9 Hz, 1H), 3.25 – 3.12 (m, 5H), 3.07 (t, *J* = 4.3 Hz, 2H), 2.28 (dt, *J*₁ = 12.5 Hz, *J*₂ = 4.1 Hz, 1H), 1.85 (s, 18H), 1.68 (q, *J* = 12.5 Hz, 1H), 0.99 (t, *J* = 7.0 Hz, 3H). ¹³C NMR (150 MHz, D₂O) δ 177.3, 110.1, 95.4, 94.8, 84.6, 81.4, 78.7, 75.3, 75.2, 73.6, 72.4, 70.0, 69.3, 68.8, 67.8, 67.5, 67.2, 60.1, 56.2, 53.1, 50.7, 49.8, 49.7, 48.2, 48.1, 40.3, 30.0, 20.8, 14.6. ESIHRMS calculated for C₂₇H₅₄N₆O₁₄Na [M+Na]⁺, 709.3596; found, 709.3571.

4'-O-Ethyl-5''-deoxy-5''-(2-hydroxyethylamino) paromomycin acetate salt (54). To a stirred solution of **52** (8 mg, 0.01 mmol) in a mixture of THF and water (1:1, 0.5 mL) was added 1M NaOH (0.25 mL) and trimethylphosphine (1M in THF, 98 μ L). After stirring for 2 h at 60 °C the reaction was concentrated to dryness under reduced pressure. The residue was purified by Sephadex C-25 column chromatography (0.8% ammonium hydroxide). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to give the desired product **54** as the peracetate salt (4.5 mg, 44%). $[\alpha]_{\text{D}}^{\text{RT}} +59.1$ (*c* 0.22, H₂O). ¹H NMR (600 MHz, D₂O) δ 5.58 (d, *J* = 3.4 Hz, 1H), 5.24 (d, *J* = 1.1 Hz, 1H), 5.11 (d, *J* = 1.4 Hz, 1H), 4.39 (dd, *J*₁ = 7.0 Hz, *J*₂ = 4.9 Hz, 1H), 4.27 (dd, *J*₁ = 4.8 Hz, *J*₂ = 1.1 Hz, 1H), 4.20 – 4.15 (m, 1H), 4.14 (t, *J* = 5.1 Hz, 1H), 4.04 (t, *J* = 3.0 Hz, 1H), 3.91 (t, *J* = 8.1 Hz, 1H), 3.90 (t, *J* = 9.5 Hz, 1H), 3.77 (t, *J* = 9.1 Hz, 1H), 3.75 – 3.63 (m, 6H), 3.63 – 3.50 (m, 3H), 3.42 – 3.32 (m, 4H), 3.28 – 3.13 (m, 5H), 3.11 – 3.08 (m, 2H), 2.28 (dt, *J*₁ = 12.6 Hz, *J*₂ = 4.1 Hz, 1H),

1.86 (s, 18H), 1.67 (q, $J = 12.6$ Hz, 1H), 1.00 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (150 MHz, D_2O) δ 177.2, 108.8, 95.1, 93.0, 82.3, 77.3, 76.3, 76.1, 75.7, 74.5, 72.6, 71.4, 70.2, 68.4, 68.3, 67.5, 67.3, 59.4, 56.3, 52.7, 50.7, 49.8, 49.8, 49.3, 48.8, 40.4, 27.6, 20.7, 14.5. ESIHRMS calculated for $\text{C}_{27}\text{H}_{55}\text{N}_6\text{O}_{14}$ $[\text{M}+\text{H}]^+$, 687.3776; found, 687.3758.

4'-O-Ethyl-6'-N-(2-hydroxyethyl)-5''-deoxy-5''-(2-hydroxyethylamino) neomycin B acetate salt (56). Compound **50** (50 mg, 0.05 mmol) was dissolved in ethanolamine (1 mL) and the solution was stirred for 18 h at RT. Then the reaction was concentrated under reduced pressure. The residue was roughly purified by column chromatography on silica gel eluting with $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (7:2.5:0.5) to give the desired product **55** (25 mg, 63%) and used for next reaction without further purification. ESIHRMS calculated for $\text{C}_{29}\text{H}_{50}\text{N}_{17}\text{O}_{14}$ $[\text{M}+\text{H}]^+$, 860.3723; found, 860.3712.

To a stirred solution of **55** (25 mg, 0.01 mmol) in a mixture of THF and water (1:1, 2 mL) was added 1M NaOH (1 mL) and trimethylphosphine (1M in THF, 0.3 mL). After stirring for 2 h at 60 $^\circ\text{C}$ the reaction was concentrated to dryness under reduced pressure. The residue was purified by Sephadex C-25 column chromatography (0.6% ammonium hydroxide). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to give the desired product **56** as the peracetate salt (18 mg, 44%). $[\alpha]_{\text{D}}^{\text{RT}} +48.9$ (c 0.9, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.53 (d, $J = 2.4$ Hz, 1H), 5.07 (br s, 1H), 4.97 (br s, 1H), 4.21 (t, $J = 5.7$ Hz, 1H), 4.11 (d, $J = 4.7$ Hz, 1H), 4.09 – 4.03 (m, 1H), 3.99 (t, $J = 5.7$ Hz, 1H), 3.96 (d, $J = 7.0$ Hz, 1H), 3.94 – 3.87 (m, 2H), 3.60 (t, $J = 9.2$ Hz, 1H), 3.58 – 3.49 (m, 5H), 3.49 – 3.35 (m, 4H), 3.34 – 3.22 (m, 4H), 3.22 – 3.16 (m, 1H), 3.13 – 2.98 (m, 5H), 2.98 – 2.92 (m, 4H), 2.79 (t, $J = 5.2$ Hz, 1H), 2.16 (dt, $J_1 = 12.6$ Hz, $J_2 = 4.1$ Hz, 1H), 1.70 (br s, 21H), 1.55 (q, $J = 12.7$ Hz, 1H), 0.85 (t, $J = 6.9$ Hz, 3H). ^{13}C NMR (150 MHz, D_2O) δ 177.0, 108.9, 95.1, 92.4, 82.4, 77.2,

76.6, 76.5, 75.2, 72.7, 71.1, 70.8, 70.0, 67.9, 67.3, 67.2, 57.3, 56.1, 56.0, 51.6, 50.6, 49.7, 49.4, 49.2, 48.4, 47.10, 41.0, 40.2, 27.5, 20.6, 14.3. ESIHRMS calculated for $C_{29}H_{59}N_7O_{14}Na$ $[M+Na]^+$, 752.4018; found, 752.3990.

1,3,6',2''',6'''-Penta-N-acetyl-neomycin B (57). A stirred solution of neomycin B sulfate salt (10 g, 14.04 mmol) in water (100 mL) was treated with conc. NH_4OH (50 mL) then concentrated under vacuum to yield neomycin free base as an off white solid. The solid was taken up in a mixture of water and methanol (3:1, 120 mL) and treated with 1N HCl (14 mL) at RT before acetic anhydride (150 mL) was added dropwise over a period of 6 h followed by stirring for 24 h. At this stage LCMS analysis of the reaction mixture showed the incomplete reaction consequently the reaction mixture was concentrated under reduced pressure and the residue was re-subjected to the identical sequence of reaction conditions, after which LCMS analysis of the reaction mixture showed penta-N-acetyl neomycin B as a major product. Finally, the reaction mixture was concentrated to dryness under reduced pressure and the residue was purified by column chromatography on silica gel eluting with $CHCl_3/MeOH/NH_4OH$ (5:4:1) to give the desired product **57** (4.5 g, 39%). $[\alpha]^{RT}_D +43.9$ (c 1.33, MeOH). 1H NMR (600 MHz, CD_3OD) δ 5.40 (d, $J = 3.4$ Hz, 1H), 5.31 (d, $J = 2.6$ Hz, 1H), 4.94 (d, $J = 1.8$ Hz, 1H), 4.26 (t, $J = 5.7$ Hz, 1H), 4.12 – 4.06 (m, 2H), 4.03 – 3.90 (m, 3H), 3.87 (t, $J = 3.2$ Hz, 1H), 3.79 (dd, $J_1 = 12.3$ Hz, $J_2 = 2.6$ Hz, 1H), 3.76 – 3.69 (m, 2H), 3.66 (t, $J = 9.1$ Hz, 1H), 3.62 (dd, $J_1 = 12.3$ Hz, $J_2 = 4.2$ Hz, 1H), 3.59 (dd, $J_1 = 14.1$ Hz, $J_2 = 2.6$ Hz, 1H), 3.52 (t, $J = 9.7$ Hz, 1H), 3.50 – 3.36 (m, 5H), 3.30 – 3.25 (m, 1H), 3.06 (t, $J = 9.4$ Hz, 1H), 2.56 (dd, $J_1 = 10.1$ Hz, $J_2 = 3.5$ Hz, 1H), 2.01 (s, 3H), 1.97 (s, 3H), 1.96 (s, 6H), 1.94 (s, 3H), 1.90 (dt, $J_1 = 12.9$ Hz, $J_2 = 4.3$ Hz, 1H), 1.40 (q, $J = 12.8$ Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.8, 172.5, 172.0, 171.9, 171.6, 108.3, 98.4, 98.1, 84.8, 81.7, 76.7, 76.3, 74.6, 74.3, 73.1, 72.9, 71.7, 71.0, 69.9, 68.0, 60.5, 56.1, 51.2, 48.4, 48.3, 40.4,

39.9, 33.3, 21.9, 21.7, 21.4, 21.3, 21.2. ESIHRMS calculated for $C_{33}H_{57}N_6O_{18}$ $[M+H]^+$, 825.3729; found, 825.3737.

1,3,6',2'',6'''-Penta-*N*-acetyl-2'-*N*-benzyl-2'-*N*-methyl-6,3',4',2'',5'',3''',4'''-hepta-*O*-acetyl-neomycin B (58). To a stirred solution of **57** (400 mg, 0.485 mmol) in methanol (8 mL) was added benzaldehyde (74 μ L, 0.73 mmol) at RT. After stirring for 0.5 h at RT glacial acetic acid (83 μ L, 1.45 mmol) and sodium cyanoborohydride (92 mg, 1.45 mmol) were added and stirring was continued for 5 h after which 4Å-MS (1.2 g) were added followed by 37% formaldehyde solution (0.2 mL), glacial acetic acid (83 μ L, 1.45 mmol) and sodium cyanoborohydride (92 mg, 1.45 mmol) at RT and stirring was continued for 2 h. The reaction was quenched with aq. $NaHCO_3$ (10 mL) at RT. After stirring for 0.5 h the reaction mixture was concentrated to dryness under reduced pressure, dissolved in methanol (50 mL), and the precipitate was filtered off. The filtrate was concentrated under reduced pressure. The residue was dissolved in pyridine (8 mL) and treated with acetic anhydride (8 mL) at RT. The resulting mixture was stirred for 18 h before it was concentrated to dryness under reduced pressure and the residue was purified by column chromatography on silica gel eluting with $CHCl_3/MeOH$ (9:1) to give the desired product **58** (370 mg, 62%). $[\alpha]^{RT}_D +59.3$ (c 1.43, MeOH). 1H NMR (600 MHz, CD_3OD) δ 7.38 (d, $J = 7.4$ Hz, 2H), 7.27 (t, $J = 7.6$ Hz, 2H), 7.19 (t, $J = 7.3$ Hz, 1H), 6.01 (d, $J = 3.2$ Hz, 1H), 5.46 (d, $J = 2.1$ Hz, 1H), 5.44 (dd, $J_1 = 11.0$ Hz, $J_2 = 9.1$ Hz, 1H), 4.94 (dd, $J_1 = 5.0$ Hz, $J_2 = 2.2$ Hz, 1H), 4.91 (t, $J = 2.9$ Hz, 1H), 4.82 – 4.79 (m, 2H), 4.77 – 4.72 (m, 2H), 4.40 (dd, $J_1 = 11.9$ Hz, $J_2 = 1.9$ Hz, 1H), 4.35 (t, $J = 5.6$ Hz, 1H), 4.22 (td, $J_1 = 6.0$ Hz, $J_2 = 2.1$ Hz, 1H), 4.13 (dd, $J_1 = 11.9$ Hz, $J_2 = 5.7$ Hz, 1H), 4.11 – 4.04 (m, 4H), 3.98 – 3.89 (m, 3H), 3.68 (d, $J = 14.1$ Hz, 1H), 3.59 (dd, $J_1 = 10.1$ Hz, $J_2 = 8.7$ Hz, 1H), 3.42 – 3.35 (m, 3H), 3.26 (dd, $J_1 = 14.2$ Hz, $J_2 = 2.9$ Hz, 1H), 2.96 (dd, $J_1 = 11.2$ Hz, $J_2 = 3.2$ Hz, 1H), 2.35 (s, 3H), 2.12 (s, 6H), 2.08 (s, 3H), 2.07 (6H),

2.02 (s, 3H), 2.01 (s, 6H), 1.99 (s, 3H), 1.97 (s, 3H), 1.94 (s, 3H), 1.86 (s, 3H), 1.82 (dt, $J_1 = 12.8$ Hz, $J_2 = 4.4$ Hz, 1H), 1.66 (q, $J = 12.8$ Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.5, 172.3, 171.7, 171.6, 171.4, 171.1, 170.6, 170.2, 170.1, 169.7, 168.7, 140.4, 128.2, 127.7, 126.3, 107.0, 98.1, 96.2, 82.5, 79.8, 76.2, 76.0, 74.4, 73.6, 72.2, 71.5, 70.0, 68.5, 66.8, 65.9, 64.6, 64.2, 59.1, 47.8, 47.7, 47.5, 39.6, 39.0, 39.0, 32.4, 21.7, 21.6, 21.4, 21.3, 21.3, 21.1, 20.0, 19.9, 19.7, 19.4, 19.3, 19.3, 19.2. ESIHRMS calculated for $\text{C}_{55}\text{H}_{78}\text{N}_6\text{O}_{25}\text{Na}$ $[\text{M}+\text{Na}]^+$, 1245.4914; found, 1245.4906.

2'-N-Methyl-neomycin B acetate salt (59). To a stirred suspension of Pd/C (70 mg) in 10% AcOH (1.5 mL) was added a solution of **58** (130 mg, 0.11 mmol) in MeOH (3 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 2 h, filtered and concentrated under reduced pressure. The residue was dissolved in aq. $\text{Ba}(\text{OH})_2$ (3 mL) and heated to reflux for 72 h. The reaction mixture was acidified with 10% AcOH and then purified by Sephadex C-25 column chromatography (1.2% NH_4OH). The product-containing fractions were concentrated under reduced pressure and the residue was dissolved in 10% AcOH and freeze dried to give the desired product **59** as the peracetate salt (30 mg, 29%). $[\alpha]_{\text{D}}^{\text{RT}} +41.4$ (c 0.29, H_2O). ^1H NMR (600 MHz, D_2O) δ 6.02 (d, $J = 3.6$ Hz, 1H), 5.20 (d, $J = 2.0$ Hz, 1H), 5.02 (br s, 1H), 4.24 (d, $J = 5.6$ Hz, 1H), 4.11 (dd, $J_1 = 4.4$ Hz, $J_2 = 2.3$ Hz, 1H), 4.04 (t, $J = 5.0$ Hz, 1H), 3.98 – 3.92 (m, 2H), 3.91 – 3.84 (m, 2H), 3.74 (t, $J = 9.0$ Hz, 1H), 3.67 – 3.62 (m, 1H), 3.61 (dd, $J_1 = 12.5$ Hz, $J_2 = 3.0$ Hz, 1H), 3.55 (br s, 1H), 3.50 – 3.44 (m, 2H), 3.34 – 3.27 (m, 2H), 3.22 (t, $J = 9.2$ Hz, 1H), 3.25 – 3.12 (m, 3H), 3.12 – 3.06 (m, 2H), 3.03 (dd, $J_1 = 13.6$ Hz, $J_2 = 7.1$ Hz, 1H), 2.64 (s, 3H), 2.21 (dt, $J_1 = 12.7$ Hz, $J_2 = 4.1$ Hz, 1H), 1.77 (s, 18H), 1.62 (q, $J = 9.2$ Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 177.3, 109.1, 95.4, 92.9, 83.9, 81.7, 75.6, 73.7, 73.5, 72.2, 70.4, 70.0, 69.3, 67.7, 67.4, 67.2, 60.0,

59.9, 50.7, 49.5, 48.6, 40.3, 39.8, 31.9, 27.8, 20.8. ESIHRMS calculated for $C_{24}H_{49}N_6O_{13}$ $[M+H]^+$, 629.3358; found, 629.3331.

1,3,6',2'',6'''-Penta-*N*-acetyl-2'-*N*-ethyl neomycin B (60). To a stirred solution of **57** (150 mg, 0.18 mmol) in methanol (5 mL) was added a 1M solution of acetaldehyde in DCM (0.3 mL, 0.3 mmol) at RT. After stirring for 0.5 h at RT glacial acetic acid (31 μ L, 0.55 mmol) and sodium cyanoborohydride (34 mg, 0.55 mmol) were added to the reaction mixture and stirring was continued for 2 h. The reaction was quenched with aq. $NaHCO_3$ (1 mL) at RT. After stirring for 0.5 h the reaction mixture was concentrated to dryness under reduced pressure and the residue was purified by column chromatography on silica gel eluting with $CHCl_3/MeOH/NH_4OH$ (6:3:1) to give the desired product **60** (90 mg, 58%). $[\alpha]^{RT}_D +58.1$ (c 0.94, MeOH). 1H NMR (600 MHz, CD_3OD) δ 5.44 (d, $J = 2.1$ Hz, 1H), 5.26 (d, $J = 2.5$ Hz, 1H), 4.95 (d, $J = 1.7$ Hz, 1H), 4.30 (t, $J = 5.4$ Hz, 1H), 4.11 (dd, $J_1 = 4.7$ Hz, $J_2 = 2.7$ Hz, 1H), 4.11 – 4.08 (m, 1H), 4.04 – 3.97 (m, 2H), 3.96 – 3.91 (m, 1H), 3.87 (t, $J = 3.3$ Hz, 1H), 3.83 (dd, $J_1 = 12.3$ Hz, $J_2 = 2.4$ Hz, 1H), 3.77 – 3.66 (m, 2H), 3.66 – 3.59 (m, 4H), 3.55 (t, $J = 9.4$ Hz, 1H), 3.52 – 3.43 (m, 3H), 3.40 (dd, $J_1 = 13.8$ Hz, $J_2 = 8.2$ Hz, 1H), 3.26 (dd, $J_1 = 14.2$, $J_2 = 6.5$ Hz, 1H), 3.08 (t, $J = 9.3$ Hz, 1H), 2.94 – 2.80 (m, 2H), 2.60 (d, $J = 10.0$ Hz, 1H), 2.02 (s, 3H), 1.97 (s, 3H), 1.96 (s, 6H), 1.94 (s, 3H), 1.90 (dt, $J_1 = 12.8$ Hz, $J_2 = 4.2$ Hz, 1H), 1.41 (q, $J = 12.7$ Hz, 1H), 1.16 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.7, 172.5, 172.0, 171.9, 171.7, 108.5, 98.5, 96.3, 85.5, 82.0, 77.4, 76.6, 74.5, 74.2, 73.1, 72.0, 71.0, 69.9, 68.0, 61.9, 60.5, 51.2, 49.8, 48.6, 48.5, 41.5, 40.5, 39.9, 33.1, 21.9, 21.7, 21.4, 21.3, 21.2, 13.3. ESIHRMS calculated for $C_{35}H_{61}N_6O_{18}$ $[M+H]^+$, 853.4042; found, 853.4028.

2'-*N*-Ethyl-neomycin B acetate salt (61). Compound **60** (75 mg, 0.09 mmol) was dissolved in aq. $Ba(OH)_2$ (2 mL) and heated to reflux for 24 h. The reaction mixture was acidified with 10% AcOH and then purified by Sephadex C-25 column chromatography (0.8% NH_4OH). The product-

containing fractions were concentrated under reduced pressure and the residue was dissolved in 10% AcOH and freeze dried to give the desired product **61** as the peracetate salt (22 mg, 25%). $[\alpha]_{\text{D}}^{\text{RT}} + 32.3$ (c 0.7, H₂O). ¹H NMR (600 MHz, D₂O) δ 6.07 (d, J = 3.7 Hz, 1H), 5.23 (d, J = 2.4 Hz, 1H), 5.06 (d, J = 1.6 Hz, 1H), 4.27 (t, J = 5.5 Hz, 1H), 4.15 (dd, J_1 = 4.8 Hz, J_2 = 2.5 Hz, 1H), 4.09 – 4.05 (m, 1H), 4.01 – 3.97 (m, 2H), 3.92 (t, J = 9.6 Hz, 1H), 3.89 (dd, J_1 = 10.5 Hz, J_2 = 8.8 Hz, 1H), 3.77 (t, J = 9.0 Hz, 1H), 3.68 – 3.62 (m, 2H), 3.60 – 3.57 (m, 1H), 3.52 – 3.47 (m, 2H), 3.37 – 3.30 (m, 2H), 3.29 – 3.12 (m, 6H), 3.11 (dd, J_1 = 8.4 Hz, J_2 = 4.2 Hz, 1H), 3.07 (dd, J_1 = 13.4, J_2 = 6.9 Hz, 2H), 2.25 (dt, J_1 = 12.6, J_2 = 4.1 Hz, 1H), 1.81 (s, 18H), 1.65 (q, J = 12.7 Hz, 1H), 1.10 (t, J = 7.3 Hz, 3H). ¹³C NMR (150 MHz, D₂O) δ 177.0, 109.3, 95.4, 92.7, 84.1, 81.8, 75.7, 73.7, 73.0, 72.2, 70.4, 70.0, 69.3, 67.7, 67.5, 67.2, 60.2, 58.4, 50.7, 49.5, 48.6, 41.9, 40.3, 39.9, 27.8, 20.6, 10.6. ESIHRMS calculated for C₂₅H₅₁N₆O₁₃ [M+H]⁺, 643.3514; found, 643.3512.

1,3,6',2'',6'''-Penta-*N*-acetyl-2'-*N*-formyl-6,3',4',2'',5'',3'',4'''-hepta-*O*-acetyl

neomycin B (62). A stirred solution of **57** (500 mg, 0.61 mmol) in DMF (10 mL) was treated with pyridine (0.15 mL) at RT and cooled to 0 °C before acetic formic anhydride (72 μ L, 0.91 mmol) was added. The reaction mixture was stirred for 4 h at 0 °C before it was quenched with excess of MeOH (10 mL). Then the reaction mixture was concentrated to dryness under reduced pressure and the residue was dissolved in pyridine (5 mL) and treated with acetic anhydride (5 mL) at RT. The resulting mixture was stirred for 18 h before it was concentrated to dryness under reduced pressure and the residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH (9:1) to give the desired product **62** (450 mg, 65%). $[\alpha]_{\text{D}}^{\text{RT}} + 37.6$ (c 1.33, MeOH). ¹H NMR (600 MHz, CD₃OD) δ 8.06 (s, 1H), 5.74 (d, J = 3.6 Hz, 1H), 5.31 (d, J = 2.7 Hz, 1H), 5.10 (dd, J_1 = 10.5 Hz, J_2 = 9.6 Hz, 1H), 4.96 – 4.71 (m, 6H), 4.57 (br s, 1H), 4.37 – 4.18 (m, 4H), 4.16 – 3.95 (m, 6H), 3.61 (d, J = 9.5 Hz, 1H), 3.47 – 3.30 (m, 4H), 2.14 (s, 3H), 2.12 (s, 3H), 2.11

(s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.97 (s, 3H), 1.93 (s, 3H), 1.89 – 1.86 (m, 4H), 1.63 (q, $J = 12.8$ Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.5, 172.3, 171.7, 171.6, 171.5, 171.4, 170.6, 170.4, 170.0, 169.8, 168.8, 162.3, 107.3, 98.0, 95.6, 82.7, 79.6, 76.7, 75.8, 75.1, 74.6, 72.2, 72.2, 71.0, 69.7, 68.5, 67.7, 65.9, 62.7, 49.6, 47.8, 47.6, 39.3, 39.0, 32.5, 21.7, 21.4, 21.3, 21.2, 21.1, 20.0, 19.8, 19.4, 19.3, 19.3, 19.3, 19.2. ESIHRMS calculated for $\text{C}_{48}\text{H}_{70}\text{N}_6\text{O}_{26}\text{Na}$ $[\text{M}+\text{Na}]^+$, 1169.4237; found, 1169.4248.

2'-Deamino neomycin B acetate salt (64). A stirred solution of **62** (400 mg, 0.35 mmol) in DCM (8 mL) was treated with Et_3N (2 mL) at RT before POCl_3 (0.3 mL, 3.49 mmol) was added dropwise. The reaction mixture was stirred for 4 h at RT before it was quenched with aq. NaHCO_3 (5 mL). Then it was concentrated to dryness under reduced pressure and the residue was dissolved in acetone (50 mL). The precipitate was filtered off and the filtrate was concentrated under reduced pressure. The residue was dissolved in a mixture of toluene and MeCN (2:1, 16 mL) and the solution was degassed by sparging with argon before tris(trimethylsilyl)silane (1 mL, 3.5 mmol) was added at RT. Then the reaction temperature was raised to 90°C and to this heated solution was added a solution of AIBN (12 mg, 0.07 mmol) in MeCN (1 mL) dropwise. The resulting mixture was stirred for 2 h at 90°C before it was concentrated to dryness under reduced pressure and the residue was purified by column chromatography on silica gel eluting with $\text{CHCl}_3/\text{MeOH}$ (9:1) to give crude compound **63** (170 mg, 44%) that was used for next reaction without further purification and characterization.

Crude compound **64** (100 mg, 0.09 mmol) was dissolved in aq. $\text{Ba}(\text{OH})_2$ (2 mL) and heated to reflux for 24 h. The reaction mixture was acidified with 10% AcOH and then purified by Sephadex C-25 column chromatography (0.8% NH_4OH). The product-containing fractions were concentrated under reduced pressure and the residue was dissolved in 10% AcOH and freeze dried.

to give the desired product **35** as the peracetate salt (22 mg, 28%). $[\alpha]_{\text{D}}^{\text{RT}} +23.2$ (*c* 0.43, H₂O). ¹H NMR (600 MHz, D₂O) δ 5.42 (d, *J* = 1.7 Hz, 1H), 5.13 (d, *J* = 3.4 Hz, 1H), 5.07 (d, *J* = 1.4 Hz, 1H), 4.26 (t, *J* = 5.3 Hz, 1H), 3.10 – 4.06 (m, 2H), 4.01 (t, *J* = 3.1 Hz, 1H), 3.96 (q, *J* = 9.6 Hz, 1H), 3.77 – 3.74 (m, 2H), 3.70 – 3.64 (m, 2H), 3.62 – 3.57 (m, 2H), 3.56 – 3.51 (m, 2H), 3.47 (t, *J* = 9.8 Hz, 1H), 3.37 (br s, 1H), 3.26 – 3.17 (m, 3H), 3.15 (dd, *J*₁ = 7.6 Hz, *J*₂ = 3.4 Hz, 1H), 3.14 – 3.10 (m, 2H), 3.08 (dd, *J*₁ = 13.6 Hz, *J*₂ = 6.4 Hz, 1H), 2.25 (dt, *J*₁ = 12.5 Hz, *J*₂ = 4.2 Hz, 1H), 2.15 – 2.11 (m, 1H), 1.83 (s, 15H), 1.63 (q, *J* = 12.6 Hz, 1H), 1.58 – 1.51 (m, 1H). ¹³C NMR (150 MHz, D₂O) δ 177.2, 108.3, 98.4, 95.7, 82.5, 81.7, 76.4, 76.3, 73.2, 72.0, 71.6, 70.1, 69.7, 67.5, 67.0, 66.8, 60.8, 50.7, 49.7, 48.6, 40.3, 40.1, 35.9, 27.8, 20.7. ESIHRMS calculated for C₂₃H₄₆N₅O₁₃ [M+H]⁺, 600.3092; found, 600.3076.

1,3,2'',6'''-Tetra-*N*-acetyl paromomycin (65). A stirred solution of paromomycin sulfate salt (10 g, 14.02 mmol) in water (100 mL) was treated with conc. NH₄OH (50 mL) then concentrated under vacuum to yield paromomycin free base as an off white solid. Then the stirred solution of paromomycin free base in a mixture of water and methanol (2:1, 150 mL) was treated with 1N HCl (14 mL) at RT before acetic anhydride (150 mL) was added dropwise over a period of 6 h and stirring was continued for additional 18 h. LCMS analysis of the reaction mixture showed the incomplete reaction. Then the reaction mixture was concentrated under reduced pressure and the residue was re-subjected to the identical reaction conditions. After this LCMS analysis of the reaction mixture showed tetra-*N*-acetyl paromomycin as a major product. Then the reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH/NH₄OH (5:4:1) to give the desired product **65** (4.5 g, 41%). $[\alpha]_{\text{D}}^{\text{RT}} +53.2$ (*c* 0.5, MeOH). ¹H NMR (600 MHz, CD₃OD) 5.47 (d, *J* = 3.6 Hz, 1H), 5.34 (d, *J* = 2.3 Hz, 1H), 4.94 (d, *J* = 1.6 Hz, 1H), 4.29 – 4.27 (t, *J* = 5.6 Hz, 1H),

4.11 (dd, $J_1 = 4.9$ Hz, $J_2 = 2.4$ Hz, 1H), 4.09 (br s, 1H), 3.98 – 3.91 (m, 3H), 3.87 (t, $J = 3.2$ Hz, 1H), 3.83 (d, $J = 9.4$ Hz, 1H), 3.78 (dd, $J_1 = 12.3$ Hz, $J_2 = 2.5$ Hz, 1H), 3.76 – 3.70 (m, 1H), 3.70 – 3.59 (m, 5H), 3.50 (dd, $J_1 = 13.9$ Hz, $J_2 = 5.0$ Hz, 1H), 3.49 (br s, 1H), 3.43 (t, $J = 9.5$ Hz, 1H), 3.42 (t, $J = 9.6$ Hz, 1H), (dd, $J_1 = 13.6$ Hz, $J_2 = 8.4$ Hz, 1H), 3.20 (t, $J = 9.3$ Hz, 1H), 2.60 (dd, $J_1 = 10.2$ Hz, $J_2 = 3.6$ Hz, 1H), 1.98 (s, 3H), 1.96 (s, 6H), 1.94 (s, 3H), 1.92 (dt, $J_1 = 12.7$ Hz, $J_2 = 4.2$ Hz, 1H), 1.41 (q, $J = 12.7$ Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.6, 172.0, 171.9, 171.6, 108.3, 98.3, 98.0, 85.2, 81.5, 76.4, 76.2, 74.8, 74.3, 73.3, 73.1, 73.1, 70.4, 69.9, 68.0, 61.5, 60.4, 55.8, 51.2, 48.4, 48.4, 39.9, 33.2, 21.9, 21.7, 21.4, 21.3. ESIHRMS calculated for $\text{C}_{31}\text{H}_{54}\text{N}_5\text{O}_{18}$ $[\text{M}+\text{H}]^+$, 784.3464; found, 784.3436.

1,3,2'',6'''-Tetra-*N*-acetyl-2'-*N*-benzyloxycarbonyl-6,3',4',6',2'',5'',3'',4'''-octa-*O*-acetyl paromomycin (66). A stirred solution of **65** (3.5 g, 4.5 mmol) in a mixture of methanol and water (4:1, 50 mL) was treated with Na_2CO_3 (1.9 g, 17.9 mmol) at RT and cooled to 0 $^\circ\text{C}$ before benzylchloroformate (1.0 mL, 6.7 mmol) was added. The reaction was slowly allowed to warm to RT and stirring was continued for 18 h. Then the reaction mixture was concentrated to dryness under reduced pressure, dissolved in methanol (100 mL), the precipitate was filtered off, and the filtrate was concentrated under reduced pressure. The residue was dissolved in pyridine (10 mL) and acetic anhydride (10 mL) was added at RT. After stirring for 18 h at RT the reaction mixture was concentrated to dryness under vacuum. The residue was purified by column chromatography on silica gel eluting with $\text{CHCl}_3/\text{MeOH}$ (19:1) to give the desired product **66** (3.2 g, 57%). $[\alpha]_D^{RT} +59.7$ (c 0.33, MeOH). ^1H NMR (600 MHz, CD_3OD) δ 7.40 – 7.25 (m, 5H), 5.67 (d, $J = 3.5$ Hz, 1H), 5.30 (d, $J = 2.9$ Hz, 1H), 5.12 – 5.02 (m, 3H), 5.01 (t, $J = 9.7$ Hz, 1H), 4.91 – 4.85 (m, 3H), 4.72 (br s, 2H), 4.27 (dd, $J_1 = 12.5$ Hz, $J_2 = 2.8$ Hz, 1H), 4.23 – 4.19 (m, 2H), 4.12 (dd, $J_1 = 12.4$ Hz, $J_2 = 2.2$ Hz, 1H), 4.07 – 3.94 (m, 8H), 3.89 (td, $J_1 = 10.4$ Hz, $J_2 = 3.7$ Hz, 1H), 3.58 (t, $J = 9.5$

Hz, 1H), 3.40 (dd, $J_1 = 13.7$ Hz, $J_2 = 6.4$ Hz, 1H), 3.29 (dd, $J_1 = 13.3$ Hz, $J_2 = 7.2$ Hz, 1H), 2.12 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.01 (dt, $J_1 = 12.8$ Hz, $J_2 = 3.5$ Hz, 1H), 1.97 (s, 3H), 1.96 (s, 3H), 1.95 (s, 6H), 1.86 (s, 3H), 1.85 (s, 3H), 1.63 (q, $J = 12.8$ Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.2, 171.6, 171.5, 171.4, 171.3, 170.9, 170.5, 170.5, 170.4, 169.7, 169.7, 168.7, 156.9, 137.0, 128.1, 127.6, 127.4, 107.2, 98.1, 97.0, 82.7, 79.3, 78.5, 76.0, 74.8, 74.5, 72.1, 71.3, 68.5, 68.5, 67.7, 66.1, 65.8, 62.3, 61.2, 53.5, 48.4, 48.2, 38.9, 32.5, 21.7, 21.3, 21.3, 21.1, 21.1, 19.9, 19.6, 19.3, 19.3, 19.3, 19.2, 19.2, 19.2. ESIHRMS calculated for $\text{C}_{55}\text{H}_{75}\text{N}_5\text{O}_{28}\text{Na}$ $[\text{M}+\text{Na}]^+$, 1276.4496; found, 1276.4452.

5-*O*-[3-*O*-(2,6-Di-*N*-acetyl-2,6-dideoxy-3,4-di-*O*-acetyl- α -L-idopyranosyl)-2,5-*O*-acetyl- β -D-ribofuranosyl]-1,3-di-*N*-benzyloxycarbonyl-6-*O*-acetyl-2-deoxystreptamine (67).

To a stirred suspension of Pd/C (800 mg) in 10% AcOH (25 mL) was added a solution of **9** (3.2 g, 2.5 mmol) in MeOH (25 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 2 h, filtered, and concentrated under reduced pressure. The residue was dissolved in 5% AcOH (25 mL) and cooled to 0 $^{\circ}\text{C}$ before NaNO_2 (880 mg, 12.7 mmol) was added slowly portion wise. The reaction was slowly allowed to warm to RT and stirring was continued for 18 h. Then the reaction was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $\text{CHCl}_3/\text{MeOH}$ (9:1) to give the desired product **67** (1.1 g, 52%). $[\alpha]_{\text{D}}^{25} +29.7$ (c 0.66, MeOH). ^1H NMR (600 MHz, CD_3OD) δ 5.21 (br s, 1H), 5.05 (d, $J = 4.9$ Hz, 1H), 4.91 (t, $J = 3.0$ Hz, 1H), 3.43 – 3.31 (m, 1H), 4.81 (t, $J = 10.0$ Hz, 1H), 4.74 (br s, 1H), 4.45 (dd, $J_1 = 7.5$ Hz, $J_2 = 4.9$ Hz, 1H), 4.41 (dd, $J_1 = 12.0$ Hz, $J_2 = 2.3$ Hz, 1H), 4.29 (dd, $J_1 = 12.0$ Hz, $J_2 = 6.2$ Hz, 1H), 4.20 – 4.15 (m, 1H), 4.11 – 4.07 (m, 1H), 4.07 – 3.97 (m, 2H), 3.82 – 3.75 (m, 1H), 3.61 (t, $J = 9.3$ Hz, 1H), 3.43 – 3.31 (m, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.05 – 3.31 (m, 1H), 2.03 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H),

1.93 (s, 3H), 1.85 (s, 3H), 1.48 (q, $J = 12.7$ Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.3, 171.8, 171.7, 171.4, 171.3, 170.7, 170.4, 169.7, 168.8, 106.9, 97.6, 82.9, 79.5, 75.5, 75.2, 74.2, 73.1, 72.1, 68.5, 66.0, 63.9, 49.5, 47.7, 47.5, 38.9, 32.6, 21.2, 21.2, 21.1, 21.1, 19.5, 19.5, 19.3, 19.2, 19.1. ESIHRMS calculated for $\text{C}_{35}\text{H}_{52}\text{N}_4\text{O}_{19}\text{Na}$ $[\text{M}+\text{Na}]^+$, 855.3123; found, 855.3137.

1,3,2''',6'''-Tetra-*N*-acetyl-2'-benzyloxy-3',4'-di-*O*-benzyl-6'-azido-6,2'',5'',3'',4'''-penta-*O*-acetyl-2',6'-dideamino neomycin B (69). A mixture of thioglycoside **68** (273 mg, 0.48 mmol) and freshly activated molecular sieves (AW300, 700 mg) was suspended in DCM (5 mL). Then DMF (112 μL , 1.44 mmol) was added and the resulting mixture was stirred for 0.25 h at RT before it was cooled to 0 $^\circ\text{C}$ and stirred for 0.25 h before NIS (110 mg, 0.48 mmol) and TMSOTf (87 μL , 0.48 mmol) were added. After stirring for an additional 1 h at 0 $^\circ\text{C}$ a solution of glycosyl acceptor **67** (200 mg, 0.24 mmol) in DCM (2.5 mL) was added and the reaction mixture was slowly allowed to warm to RT and stirring was continued for 24 h. Then the reaction was quenched with aq. $\text{Na}_2\text{S}_2\text{O}_3$ (2 mL) and concentrated to dryness under reduced pressure. The residue was dissolved in MeOH (20 mL), the precipitate was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $\text{CHCl}_3/\text{MeOH}$ (19:1) to give the desired product **69** (90 mg, 29%). $[\alpha]_{\text{D}}^{\text{RT}} +86.0$ (c 0.83, MeOH). ^1H NMR (600 MHz, CD_3OD) δ 7.41 – 7.16 (m, 15H), 6.05 (d, $J = 3.3$ Hz, 1H), 5.39 (br s, 1H), 5.01 (d, $J = 4.6$ Hz, 1H), 4.93 – 4.79 (m, 5H), 4.78 (d, $J = 1.6$ Hz, 1H), 4.74 (d, $J = 11.2$ Hz, 1H), 4.73 (s, 1H), 4.69 (d, $J = 11.2$ Hz, 1H), 4.56 (d, $J = 11.1$ Hz, 1H), 4.49 (d, $J = 9.8$ Hz, 1H), 4.32 (dd, $J_1 = 6.6$ Hz, $J_2 = 4.8$ Hz, 1H), 4.12 – 4.04 (m, 4H), 4.03 – 3.97 (m, 2H), 3.96 – 3.87 (m, 3H), 3.74 (t, $J = 9.3$ Hz, 1H), 3.53 (dd, $J_1 = 13.1$ Hz, $J_2 = 1.7$ Hz, 1H), 3.47 (dd, $J_1 = 9.8$ Hz, $J_2 = 3.5$ Hz, 1H), 3.46 – 3.38 (m, 3H), 3.31 (dd, $J_1 = 13.5$ Hz, $J_2 = 7.4$ Hz, 1H), 2.10 (s, 6H), 2.09 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.96 (s, 6H), 1.88 (s, 3H), 1.86 (s, 3H), 1.82 (dt, $J_1 = 12.9$ Hz,

$J_2 = 4.4$ Hz, 1H), 1.73 (q, $J = 12.8$ Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.3, 171.6, 171.4, 171.3, 171.2, 170.6, 170.5, 169.7, 168.7, 138.7, 138.6, 138.3, 127.9, 127.8, 127.5, 127.4, 127.4, 127.3, 127.2, 127.1, 107.5, 97.7, 94.6, 82.5, 81.3, 80.1, 79.3, 78.2, 76.0, 75.8, 75.6, 75.2, 74.6, 74.0, 73.1, 72.3, 70.6, 68.5, 65.9, 63.7, 51.2, 47.9, 47.7, 47.5, 39.0, 32.3, 22.0, 21.35, 21.3, 21.1, 20.0, 19.6, 19.4, 19.3, 19.2. ESIHRMS calculated for $\text{C}_{62}\text{H}_{79}\text{N}_7\text{O}_{23}\text{Na}$ $[\text{M}+\text{Na}]^+$, 1312.5125; found, 1312.5109.

2'-Deamino-2'-hydroxy neomycin B acetate salt (70). To a stirred suspension of Pd/C (90 mg) in 10% AcOH (1.5 mL) was added a solution of **69** (45 mg, 0.03 mmol) in dioxane (1.5 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (45 atm) for 12 h, filtered and concentrated under reduced pressure. The residue was dissolved in 2.5M NaOH (1.5 mL) and heated to reflux for 3 h. The reaction mixture was acidified with 10% AcOH and then purified by Sephadex C-25 column chromatography (1.0% NH_4OH). The product-containing fractions were concentrated under reduced pressure and the residue was dissolved in 10% AcOH and freeze dried to give the desired product **70** as the peracetate salt (11 mg, 34%). $[\alpha]_{\text{D}}^{\text{RT}} +35.3$ (c 0.73, H_2O). $[\alpha]_{\text{D}}^{\text{RT}} +59.7$ (c 0.31, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.43 (d, $J = 3.9$ Hz, 1H), 5.13 (d, $J = 2.1$ Hz, 1H), 5.02 (br s, 1H), 4.26 (t, $J = 5.6$ Hz, 1H), 4.11 (dd, $J_1 = 4.7$ Hz, $J_2 = 2.4$ Hz, 1H), 4.06 – 4.01 (m, 1H), 3.96 (t, $J = 2.9$ Hz, 1H), 3.95 – 3.90 (m, 1H), 3.76 (t, $J = 9.7$ Hz, 1H), 3.69 – 3.62 (m, 2H), 3.60 (dd, $J_1 = 12.4$ Hz, $J_2 = 3.1$ Hz, 1H), 3.54 (br s, 1H), 3.52 (t, $J = 9.6$ Hz, 1H), 3.49 (dd, $J_1 = 12.6$ Hz, $J_2 = 5.1$ Hz, 1H), 3.46 (t, $J = 9.8$ Hz, 1H), 3.38 (dd, $J_1 = 10.0$ Hz, $J_2 = 3.9$ Hz, 1H), 3.34 – 3.27 (m, 2H), 3.19 – 3.05 (m, 5H), 3.00 (dd, $J_1 = 13.6$ Hz, $J_2 = 6.2$ Hz, 1H), 2.21 (dt, $J_1 = 12.7$ Hz, $J_2 = 4.1$ Hz, 1H), 1.78 (s, 15H), 1.60 (dd, $J = 12.7$ Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 177.0, 108.4, 97.1, 95.3, 82.3, 81.5, 75.9, 75.7, 73.3, 72.1, 71.3, 70.6, 70.4, 70.0, 68.8,

67.4, 67.0, 60.5, 50.6, 49.5, 48.4, 40.2, 40.0, 27.7, 20.5. ESIHRMS calculated for $C_{23}H_{45}N_5O_{14}Na$ $[M+Na]^+$, 638.2861; found, 638.2875.

1,3,6',2'',6'''-Penta-*N*-acetyl-1,3,6',2'',6'''-penta-*N*-*tert*-butoxycarbonyl-2'-azido-6,3',4',2'',5'',3'',4'''-hepta-*O*-acetyl-2'-deamino neomycin B (71). A stirred solution of neomycin B sulfate salt (10 g, 14.04 mmol) in water (100 mL) was treated with conc. NH_4OH (50 mL) then concentrated under vacuum to yield neomycin free base as an off white solid. This solid was taken up in a mixture of water and methanol (3:1, 120 mL) and treated with 1N HCl (14 mL) at RT before acetic anhydride (150 mL) was added dropwise over a period of 6 h and stirring was continued for additional 24 h. At this stage LCMS analysis of the reaction mixture showed incomplete reaction, consequently the reaction mixture was concentrated under reduced pressure and the residue was re-subjected to the same sequence of reaction conditions after which LCMS analysis of the reaction mixture showed penta-*N*-acetyl neomycin B as a major product. The reaction mixture was concentrated to dryness under reduced pressure and the residue was dissolved in MeOH (200 mL), the precipitate was filtered off and the filtrate was concentrated under reduced pressure. The residue was dissolved in a mixture methanol and water (2:3, 120 mL), treated with potassium carbonate (5.8 g, 42.1 mmol) at RT and cooled to 0 °C before imidazole-1-sulfonyl azide hydrochloride (4.4 g, 21.0 mmol) and copper (II) sulfate (224 mg, 1.4 mmol) were added. The reaction mixture was allowed to warm to RT and stirring was continued for 18 h after which it was concentrated to dryness under reduced pressure, dissolved in methanol (200 mL), the precipitate was filtered off and the filtrate concentrated under reduced pressure. The residue was dissolved in THF (100 mL) and $(Boc)_2O$ (45.9 g, 210.6 mmol) and DMAP (8.6 g, 70.2 mmol) were added followed by heating to reflux for 48 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue was dissolved in pyridine (50 mL) and treated with

acetic anhydride (50 mL) at RT. The resulting mixture was stirred for 18 h before it was concentrated to dryness under reduced pressure. The residue was dissolved in ethyl acetate (500 mL) and washed with water (2 x 250 mL) and brine (2 x 250 mL). The organic layer was dried over Na₂SO₄, concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel eluting with ethyl acetate/hexane (1:1) to give the desired product **71** (3 g, 13%). $[\alpha]_{\text{D}}^{\text{RT}} +56.2$ (*c* 0.4, MeOH). ESIHRMS calculated for C₇₂H₁₀₈N₈O₃₅Na [M+Na]⁺, 1667.6815; found, 1667.6802.

1,3,6',2''',6'''-Penta-*N*-*tert*-butoxycarbonyl neomycin B (72). To a stirred solution of **71** (1.1 g, 0.67 mmol) in MeOH (11 mL) was added NaOMe (867 mg, 16.1 mmol) at RT followed by stirring for 3 h. The reaction mixture was neutralized with Amberlyst (H-form), filtered, and concentrated under reduced pressure. Then the residue was dissolved in a mixture of THF and water (2:1, 15 mL) and trimethylphosphine (1M in THF, 1.3 mL) was added at RT. After stirring for 3 h at 60 °C the reaction mixture was concentrated to dryness under reduced pressure and the residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH (7:1) to give the desired product **72** (400 mg, 53%). $[\alpha]_{\text{D}}^{\text{RT}} +30.0$ (*c* 0.48, MeOH). ESIHRMS calculated for C₄₈H₈₇N₆O₂₃ [M+H]⁺, 1115.5823; found, 1115.5814.

1,3,6',2''',6'''-Penta-*N*-*tert*-butoxycarbonyl-2'-*N*-(2-azidoacetyl) neomycin B (74). To a stirred solution of **72** (150 mg, 0.13 mmol) and 2-azidoacetic acid **73** (27 mg, 0.27 mmol) in THF (1.5 mL) was added EDC.HCl (51 mg, 0.27 mmol), HOBt (36 mg, 0.27 mmol), and DIPEA (116 μL, 0.67 mmol) at RT. After stirring for 2 h at RT the reaction mixture was quenched with 1N HCl (30 mL) and extracted with DCM (3 x 30 mL). The combined DCM layer was dried over Na₂SO₄ and concentrated under reduced pressure and the residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH (9:1) to give the desired product **74** (110 mg, 68%). $[\alpha]_{\text{D}}^{\text{RT}}$

+32.3 (*c* 1.16, MeOH). ESIHRMS calculated for C₅₀H₈₇N₉O₂₄Na [M+Na]⁺, 1220.5762; found, 1220.5723.

2'-N-(2-Aminoacetyl) neomycin B acetate salt (75). To a stirred suspension of Pd/C (50 mg) in 10% AcOH (1 mL) was added a solution of **74** (100 mg, 0.08 mmol) in dioxane (2 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 2 h, filtered and concentrated under reduced pressure. The residue was dissolved in a mixture of TFA/Water/Anisole (90:7:3, 2 mL) and stirred for 1 h at RT before it was concentrated to dryness under reduced pressure. The residue was purified by Sephadex C-25 column chromatography (0.8% NH₄OH). The product-containing fractions were concentrated under reduced pressure and the residue was dissolved in 10% AcOH and freeze dried to give the desired product **75** as the peracetate salt (40 mg, 48%). $[\alpha]^{RT}_D +34.7$ (*c* 0.96, H₂O). ¹H NMR (600 MHz, D₂O) δ 5.62 (d, *J* = 3.8 Hz, 1H), 4.86 (br s, 1H), 4.85 (br s, 1H), 4.12 (dd, *J*₁ = 6.9 Hz, *J*₂ = 4.7 Hz, 1H), 4.03 (d, *J* = 4.3 Hz, 1H), 3.92 – 3.87 (m, 1H), 3.83 (t, *J* = 2.8 Hz, 1H), 3.73 (dd, *J*₁ = 10.7 Hz, *J*₂ = 3.8 Hz, 1H), 3.69 – 3.65 (m, 1H), 3.64 (t, *J* = 9.5 Hz, 1H), 3.52 – 3.41 (m, 6H), 3.40 (d, *J* = 2.0 Hz, 1H), 3.33 – 3.24 (m, 2H), 3.17 (br s, 1H), 3.14 – 2.99 (m, 4H), 2.98 – 2.90 (m, 2H), 2.86 (dd, *J*₁ = 13.5 Hz, *J*₂ = 7.1 Hz, 1H), 2.06 (dt, *J*₁ = 12.5 Hz, *J*₂ = 4.0 Hz, 1H), 1.63 (s, 18H), 1.48 (dd, *J* = 12.7 Hz, 2H). ¹³C NMR (150 MHz, D₂O) δ 177.4, 167.0, 109.4, 95.3, 94.9, 84.0, 81.0, 75.2, 73.1, 73.1, 72.0, 70.9, 70.0, 69.5, 68.7, 67.3, 66.9, 60.2, 52.3, 50.6, 49.6, 48.7, 40.3, 40.1, 40.1, 27.6, 20.9. ESIHRMS calculated for C₂₅H₅₀N₇O₁₄ [M+H]⁺, 672.3416; found, 672.3400.

1,3,2'',6''-Tetra-N-acetyl-2'-N-benzyl-2'-N-methyl paromomycin (78). To a stirred solution of **65** (500 mg, 0.42 mmol) in methanol (10 mL) was added benzaldehyde (64 μ L, 0.63 mmol) at RT. After stirring for 0.5 h at RT glacial acetic acid (48 μ L, 1.07 mmol) and sodium cyanoborohydride (68 mg, 1.07 mmol) were added and stirring was continued for 18 h after which

4Å-MS (2 g) were added followed by 37% formaldehyde solution (103 μ L, 1.26 mmol), glacial acetic acid (48 μ L, 1.07 mmol) and sodium cyanoborohydride (68 mg, 1.07 mmol) at RT and stirring was continued for 2 h. The reaction was quenched with aq. NaHCO_3 (10 mL) at RT. After stirring for 0.5 h the reaction mixture was concentrated to dryness under reduced pressure, dissolved in methanol (50 mL), and the precipitate was filtered off. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel eluting with $\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (6:3:1) to give the desired product **78** (156 mg, 42%). $[\alpha]_{\text{D}}^{\text{RT}} +36.3$ (*c* 0.27, MeOH). ^1H NMR (600 MHz, CD_3OD) δ 7.42 (d, $J = 7.4$ Hz, 2H), 7.33 (t, $J = 7.5$ Hz, 2H), 7.26 (t, $J = 7.3$ Hz, 1H), 5.80 (d, $J = 2.9$ Hz, 1H), 5.40 (br s, 1H), 4.93 (d, $J = 1.6$ Hz, 1H), 4.50 (dd, $J_1 = 7.2$ Hz, $J_2 = 4.5$ Hz, 1H), 4.12 (d, $J = 4.4$ Hz, 1H), 4.10 – 4.00 (m, 5H), 3.97 – 3.92 (m, 1H), 3.91 (dd, $J_1 = 10.8$ Hz, $J_2 = 8.5$ Hz, 1H), 4.87 (t, $J = 3.4$ Hz, 1H), 3.97 – 3.83 (m, 1H), 3.83 – 3.78 (m, 2H), 3.78 – 3.71 (m, 2H), 3.69 (dd, $J_1 = 12.1$ Hz, $J_2 = 3.1$ Hz, 1H), 3.65 (t, $J = 5.8$ Hz, 1H), 3.63 – 3.57 (m, 2H), 3.54 (dd, $J_1 = 13.9$ Hz, $J_2 = 5.1$ Hz, 1H), 3.49 (br s, 1H), 3.37 (dd, $J_1 = 13.9$ Hz, $J_2 = 8.4$ Hz, 1H), 3.24 (t, $J = 9.0$ Hz, 1H), 2.80 (dd, $J_1 = 11.0$ Hz, $J_2 = 2.9$ Hz, 1H), 2.58 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.91 – 1.86 (dt, $J_1 = 12.8$ Hz, $J_2 = 4.2$ Hz), 1.43 (q, $J = 12.7$ Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.6, 172.0, 171.9, 171.2, 138.0, 129.1, 128.0, 127.2, 104.9, 98.1, 94.9, 85.5, 81.5, 76.2, 74.4, 73.3, 73.3, 72.6, 71.8, 71.5, 69.9, 69.1, 67.9, 65.4, 61.7, 59.9, 58.8, 51.1, 49.8, 48.0, 39.8, 38.6, 33.0, 21.8, 21.6, 21.3, 21.3. ESIHRMS calculated for $\text{C}_{39}\text{H}_{62}\text{N}_5\text{O}_{18}$ $[\text{M}+\text{H}]^+$, 888.4090; found, 888.4103.

2'-N-Methyl paromomycin acetate salt (79). To a stirred suspension of $\text{Pd}(\text{OH})_2/\text{C}$ (70 mg) in MeOH (0.5 mL) was added a solution of **78** (70 mg, 0.08 mmol) in MeOH (2.0 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 1 h, filtered and concentrated under reduced pressure. The residue was dissolved in 2.5M NaOH (2 mL) and heated

to reflux for 15 h. The reaction mixture was acidified with 10% AcOH and then purified by Sephadex C-25 column chromatography (0.4% NH₄OH). The product-containing fractions were concentrated under reduced pressure and the residue was dissolved in 10% AcOH and freeze dried to give the desired product **79** as the peracetate salt (20 mg, 27%). $[\alpha]_{\text{D}}^{\text{RT}} +39.1$ (*c* 0.67, H₂O). ¹H NMR (600 MHz, D₂O) δ 5.74 (d, *J* = 3.6 Hz, 1H), 5.17 (d, *J* = 3.2 Hz, 1H), 5.08 (br s, 1H), 4.29 (t, *J* = 5.6 Hz, 1H), 4.12 – 4.05 (m, 2H), 4.01 (t, *J* = 2.9 Hz, 1H), 3.99 – 3.95 (m, 1H), 3.88 (t, *J* = 9.5 Hz, 1H), 3.78 (t, *J* = 9.6 Hz, 1H), 3.73 (t, *J* = 9.1 Hz, 1H), 3.73 – 3.69 (m, 1H), 3.66 (dd, *J*₁ = 12.3 Hz, *J*₂ = 2.7 Hz, 1H), 3.60 (d, *J* = 1.8 Hz, 1H), 3.73 – 3.69 (m, 4H), 3.42 – 3.34 (m, 2H), 3.28 (t, *J* = 9.1 Hz, 1H), 3.24 – 3.10 (m, 4H), 2.66 (s, 3H), 2.27 (dt, *J*₁ = 12.5 Hz, *J*₂ = 4.0 Hz, 1H), 1.82 (s, 15H), 1.64 (q, *J* = 12.6 Hz, 1H). ¹³C NMR (150 MHz, D₂O) δ 177.3, 109.1, 95.5, 93.6, 83.4, 81.5, 76.5, 75.3, 73.7, 73.2, 71.9, 70.1, 69.1, 68.6, 67.5, 67.1, 60.4, 60.1, 60.0, 50.7, 49.4, 49.0, 40.3, 31.8, 27.9, 20.7. ESIHRMS calculated for C₂₄H₄₈N₅O₁₄ [M+H]⁺, 630.3198; found, 630.3180.

1,3,2'',6'''-Tetra-*N*-acetyl-2'-*N*-ethyl paromomycin (80). To a stirred solution of **65** (150 mg, 0.19 mmol) in methanol (5 mL) was added a 1M solution of acetaldehyde in DCM (0.3 mL, 0.3 mmol) at RT. After stirring for 0.5 h at RT glacial acetic acid (33 μ L, 0.57 mmol) and sodium cyanoborohydride (36 mg, 0.57 mmol) were added to the reaction mixture and stirring was continued for 2 h. The reaction was quenched with aq. NaHCO₃ (1 mL) at RT. After stirring for 0.5 h the reaction mixture was concentrated to dryness under reduced pressure, and the residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH/NH₄OH (6:3:1) to give the desired product **80** (100 mg, 64%). $[\alpha]_{\text{D}}^{\text{RT}} +46.5$ (*c* 1.0, MeOH). ¹H NMR (600 MHz, CD₃OD) δ 5.51 (br s, 1H), 5.28 (d, *J* = 2.3 Hz, 1H), 4.94 (d, *J* = 1.4 Hz, 1H), 4.31 (t, *J* = 5.6 Hz, 1H), 4.12 (dd, *J*₁ = 4.3 Hz, *J*₂ = 2.3 Hz, 1H), 4.09 (br s, 1H), 4.02 – 3.98 (m, 1H), 3.97 – 3.91 (m, 2H), 3.87 (t, *J* = 3.2 Hz, 1H), 3.82 (br s, 1H), 3.80 (br s, 1H), 3.76 – 3.70 (m, 1H), 3.69 – 3.58 (m,

6H), 3.52 – 3.44 (m, 3H), 3.40 (dd, $J_1 = 13.8$ Hz, $J_2 = 8.3$ Hz, 1H), 3.25 (t, $J = 9.2$ Hz, 1H), 2.91 (br d, $J = 6.7$ Hz, 2H), 2.64 (br d, $J = 9.3$ Hz, 1H), 1.98 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.90 (dt, $J_1 = 12.8$ Hz, $J_2 = 4.3$ Hz, 1H), 1.43 (q, $J = 12.8$ Hz, 1H), 1.17 (t, $J = 7.1$ Hz, 3H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.6, 172.0, 171.9, 171.7, 108.3, 98.4, 96.2, 85.8, 81.9, 76.8, 76.3, 74.4, 74.1, 73.2, 72.9, 71.5, 70.6, 69.9, 67.9, 61.6, 61.3, 60.5, 51.1, 48.6, 48.4, 41.6, 39.8, 33.0, 21.9, 21.7, 21.4, 21.3, 13.2. ESIHRMS calculated for $\text{C}_{33}\text{H}_{58}\text{N}_5\text{O}_{18}$ $[\text{M}+\text{H}]^+$, 812.3777; found, 812.3814.

2'-N-Ethyl-paromomycin acetate salt (81). Compound **80** (90 mg, 0.11 mmol) was dissolved in 2.5M NaOH (2 mL) and heated to reflux for 9 h. The reaction mixture was acidified with 10% AcOH and then purified by Sephadex C-25 column chromatography (0.6% NH_4OH). The product-containing fractions were concentrated under reduced pressure and the residue was dissolved in 10% AcOH and freeze dried to give the desired product **81** as the peracetate salt (46 mg, 43%). $[\alpha]_{\text{D}}^{25} +50.0$ (c 0.46, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.77 (d, $J = 3.5$ Hz, 1H), 5.11 (d, $J = 2.3$ Hz, 1H), 5.00 (br s, 1H), 4.24 (t, $J = 5.7$ Hz, 1H), 4.06 – 4.03 (m, 1H), 4.03 – 4.00 (m, 1H), 3.94 (t, $J = 2.6$ Hz, 1H), 4.06 – 4.03 (m, 1H), 3.82 (t, $J = 9.5$ Hz, 1H), 3.74 (t, $J = 9.6$ Hz, 1H), 3.67 (t, $J = 9.2$ Hz, 1H), 3.63 (d, $J = 11.7$ Hz, 1H), 3.59 (dd, $J_1 = 12.4$ Hz, $J_2 = 2.4$ Hz, 1H), 3.52 (d, $J = 1.6$ Hz, 1H), 3.51 – 3.41 (m, 4H), 3.33 – 3.25 (m, 2H), 3.22 (t, $J = 9.2$ Hz, 1H), 3.17 (dd, $J_1 = 10.5$ Hz, $J_2 = 3.6$ Hz, 1H), 3.13 (dd, $J_1 = 13.7$ Hz, $J_2 = 6.8$ Hz, 1H), 3.10 – 2.97 (m, 4H), 2.19 (dt, $J_1 = 12.7$ Hz, $J_2 = 4.0$ Hz, 1H), 1.75 (s, 15H), 1.58 (q, $J = 12.7$ Hz, 1H), 1.05 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (150 MHz, D_2O) δ 177.3, 109.1, 95.3, 93.0, 83.7, 81.2, 75.1, 74.9, 73.5, 73.2, 71.8, 70.0, 69.0, 68.4, 67.4, 67.0, 60.0, 60.0, 58.6, 50.6, 49.4, 48.9, 41.7, 40.2, 27.7, 20.7, 10.4. ESIHRMS calculated for $\text{C}_{25}\text{H}_{50}\text{N}_5\text{O}_{14}$ $[\text{M}+\text{H}]^+$, 644.3354; found, 644.3347.

1,3,2'',6''-Tetra-*N*-acetyl-2'-*N*-propyl paromomycin (82). To a stirred solution of **65** (150 mg, 0.19 mmol) in methanol (5 mL) was added a 1M solution of propionaldehyde in DCM (0.3 mL, 0.3 mmol) at RT. After stirring for 0.5 h at RT glacial acetic acid (33 μ L, 0.57 mmol) and sodium cyanoborohydride (36 mg, 0.57 mmol) were added to the reaction mixture and stirring was continued for 3 h. The reaction was quenched with aq. NaHCO₃ (1 mL) at RT. After stirring for 0.5 h the reaction mixture was concentrated to dryness under reduced pressure, and the residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH/NH₄OH (6:3:1) to give the desired product **82** (110 mg, 70%). $[\alpha]_D^{RT} +38.6$ (*c* 0.65, MeOH). ¹H NMR (600 MHz, CD₃OD) δ 5.55 (br s, 1H), 5.28 (d, *J* = 2.1 Hz, 1H), 4.94 (br s, 1H), 4.32 (t, *J* = 5.5 Hz, 1H), 4.14 – 4.06 (m, 2H), 4.03 – 3.98 (m, 1H), 3.97 – 3.90 (m, 2H), 3.86 (t, *J* = 3.1 Hz, 1H), 3.83 – 3.78 (m, 2H), 3.76 – 3.70 (m, 1H), 3.69 – 3.57 (m, 6H), 3.53 – 3.44 (m, 3H), 3.39 (dd, *J*₁ = 13.8 Hz, *J*₂ = 8.3 Hz, 1H), 3.24 (t, *J* = 9.2 Hz, 1H), 2.83 (br s, 2H), 2.66 (br s, 1H), 1.97 (s, 3H), 1.96 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.90 (dt, *J*₁ = 12.8 Hz, *J*₂ = 4.2 Hz, 1H), 3.83 – 3.78 (m, 2H), 1.44 (q, *J* = 12.8 Hz, 1H), 0.95 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (150 MHz, CD₃OD) δ 172.6, 172.0, 171.9, 171.7, 108.2, 98.4, 95.8, 85.8, 81.9, 76.6, 76.5, 74.4, 74.0, 73.1, 72.8, 71.4, 70.6, 70.0, 67.9, 61.6, 61.2, 60.5, 51.0, 49.2, 48.6, 48.3, 39.7, 33.0, 22.0, 21.9, 21.7, 21.4, 21.3, 10.7. ESIHRMS calculated for C₃₄H₆₀N₅O₁₈ [M+H]⁺, 826.3933; found, 826.3929.

2'-*N*-Propyl paromomycin acetate salt (83). Compound **82** (110 mg, 0.13 mmol) was dissolved in 2.5M NaOH (2.5 mL) and heated to reflux for 15 h. The reaction mixture was acidified with 10% AcOH and then purified by Sephadex C-25 column chromatography (0.6% NH₄OH). The product-containing fractions were concentrated under reduced pressure, and the residue was dissolved in 10% AcOH and freeze dried to give the desired product **83** as the peracetate salt (63 mg, 49%). $[\alpha]_D^{RT} +54.3$ (*c* 0.6, H₂O). ¹H NMR (600 MHz, D₂O) δ 5.76 (d, *J* = 3.5 Hz, 1H), 5.07

(d, $J = 2.6$ Hz, 1H), 4.97 (br s, 1H), 4.21 (t, $J = 5.7$ Hz, 1H), 4.02 – 3.97 (m, 2H), 3.91 (t, $J = 2.9$ Hz, 1H), 3.88 – 3.84 (m, 1H), 3.80 (t, $J = 9.6$ Hz, 1H), 3.71 (t, $J = 9.6$ Hz, 1H), 3.64 – 3.58 (m, 2H), 3.56 (dd, $J_1 = 12.4$ Hz, $J_2 = 2.6$ Hz, 1H), 3.49 (d, $J = 1.4$ Hz, 1H), 3.48 – 3.38 (m, 4H), 3.29 – 3.22 (m, 2H), 3.19 (t, $J = 9.2$ Hz, 1H), 3.14 (dd, $J_1 = 10.4$ Hz, $J_2 = 3.6$ Hz, 1H), 3.11 (dd, $J_1 = 13.7$ Hz, $J_2 = 6.9$ Hz, 1H), 3.08 – 3.00 (m, 2H), 2.95 – 2.81 (m, 2H), 2.16 (dt, $J_1 = 12.6$ Hz, $J_2 = 4.1$ Hz, 1H), 1.72 (s, 15H), 1.56 (q, $J = 12.6$ Hz, 1H), 1.50 – 1.34 (m, 2H), 0.66 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (150 MHz, D_2O) δ 177.2, 109.1, 95.2, 92.9, 83.7, 81.2, 75.1, 74.8, 73.5, 73.2, 71.8, 70.0, 69.0, 68.4, 67.4, 67.0, 60.0, 59.9, 58.8, 50.6, 49.4, 48.9, 47.7, 40.2, 27.7, 20.7, 18.9, 9.9. ESIHRMS calculated for $\text{C}_{26}\text{H}_{52}\text{N}_5\text{O}_{14}$ $[\text{M}+\text{H}]^+$, 658.3511; found, 658.3502.

1,3,2'',6'''-Tetra-*N*-acetyl-2'-deamino-2'-benzyloxy-3',4',6'-tri-*O*-benzyl-6,2'',5'',3''',4'''-penta-*O*-acetyl paromomycin (85). A mixture of thioglycoside **84** (213 mg, 0.24 mmol) and freshly activated molecular sieves (AW300, 600 mg) was suspended in DCM (5 mL). Then DMF (111 μL , 1.44 mmol) was added and the resulting mixture was stirred for 0.25 h at RT before it was cooled to 0 $^\circ\text{C}$ and stirred for 0.25 h before NIS (83 mg, 0.36 mmol) and TMSOTf (65 μL , 0.36 mmol) were added. After stirring for additional 0.5 h at 0 $^\circ\text{C}$ a solution of glycosyl acceptor **67** (200 mg, 0.24 mmol) in DCM (2.5 mL) was added to the reaction mixture and the reaction mixture was slowly allowed to warm to RT and stirring was continued for 18 h. Then the reaction was quenched with aq. $\text{Na}_2\text{S}_2\text{O}_3$ (2 mL) and concentrated to dryness under reduced pressure. The residue was dissolved in MeOH (20 mL), precipitate was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $\text{CHCl}_3/\text{MeOH}$ (19:1) to give the desired product **85** (150 mg, 46 %). $[\alpha]_{\text{D}}^{\text{RT}} +134.5$ (c 0.47, MeOH). ^1H NMR (600 MHz, CD_3OD) δ 7.40 – 7.31 (m, 6H), 7.31 – 7.17 (m, 12H), 7.13 (d, $J = 6.5$ Hz, 2H), 6.01 (d, $J = 3.3$ Hz, 1H), 5.41 (br s, 1H), 5.02 (d, $J = 4.4$ Hz, 1H),

4.94 – 4.80 (m, 4H), 4.77 (d, $J = 8.8$ Hz, 2H), 4.74 (t, $J = 10.7$ Hz, 2H), 4.66 (d, $J = 11.2$ Hz, 1H), 4.57 (d, $J = 11.7$ Hz, 1H), 4.54 – 4.48 (m, 2H), 4.46 (d, $J = 10.8$ Hz, 1H), 4.34 (t, $J = 5.8$ Hz, 1H), 4.17 – 3.96 (m, 7H), 3.93 (t, $J = 8.9$ Hz, 1H), 3.89 (t, $J = 9.0$ Hz, 2H), 3.78 – 3.71 (m, 2H), 3.67 (dd, $J_1 = 10.6$ Hz, $J_2 = 4.6$ Hz, 1H), 3.51 (t, $J = 9.5$ Hz, 1H), 3.46 (dd, $J_1 = 9.7$ Hz, $J_2 = 3.3$ Hz, 1H), 3.40 (dd, $J_1 = 13.7$ Hz, $J_2 = 6.0$ Hz, 1H), 3.34 – 3.24 (m, 1H), 2.10 (s, 9H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.85 (s, 3H), 1.83 (s, 3H), 1.79 (dt, $J_1 = 12.8$ Hz, $J_2 = 4.4$ Hz, 1H), 1.65 (q, $J = 12.8$ Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 172.3, 171.6, 171.4, 171.3, 171.2, 170.6, 170.5, 169.6, 168.7, 138.7, 138.4, 137.9, 128.1, 127.9, 127.9, 127.8, 127.6, 127.5, 127.5, 127.4, 127.2, 127.1, 127.0, 107.4, 97.7, 94.7, 82.6, 81.5, 80.0, 79.2, 77.7, 76.1, 75.4, 75.4, 75.1, 74.5, 74.1, 73.4, 73.4, 73.2, 72.2, 70.9, 70.0, 68.5, 65.9, 63.5, 47.7, 47.5, 38.9, 32.4, 21.9, 21.3, 21.3, 21.1, 20.0, 19.7, 19.3, 19.2, 19.2. ESIHRMS calculated for $\text{C}_{69}\text{H}_{86}\text{N}_4\text{O}_{24}\text{Na}$ $[\text{M}+\text{Na}]^+$, 1377.5530; found, 1377.5514.

2'-Deamino-2'-hydroxy paromomycin acetate salt (86). To a stirred suspension of $\text{Pd}(\text{OH})_2/\text{C}$ (150 mg) in MeOH (0.5 mL) was added a solution of **85** (150 mg, 0.11 mmol) in MeOH (3.5 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (1 atm) for 4 h, filtered and concentrated under reduced pressure. The residue was dissolved in 2.5 M NaOH (3 mL) and heated to reflux for 8 h. The reaction mixture was acidified with 10% AcOH and then purified by Sephadex C-25 column chromatography (0.6% NH_4OH). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to give the desired product **86** as the peracetate salt (40 mg, 42 %). $[\alpha]_{\text{D}}^{\text{RT}} +35.3$ (c 0.73, H_2O). ^1H NMR (600 MHz, D_2O) δ 4.96 (d, $J = 2.6$ Hz, 1H), 4.93 (d, $J = 3.8$ Hz, 1H), 4.89 (br s, 1H), 4.12 (t, $J = 5.7$ Hz, 1H), 3.92 – 3.88 (m, 2H), 3.84 (br s, 1H), 3.79 – 3.74 (m, 1H), 3.57 – 3.51 (m, 2H), 3.50 – 3.44 (m, 2H), 3.43 – 3.25 (m, 6H), 3.24 – 3.12 (m, 3H), 3.03 (dd, $J_1 = 13.5$

Hz, $J_2 = 7.4$ Hz, 1H), 2.99 (t, $J = 9.6$ Hz, 1H), 3.99 – 2.91 (m, 2H), 2.09 (dt, $J_1 = 12.6$ Hz, $J_2 = 4.1$ Hz, 1H), 1.64 (s, 12H), 1.46 (q, $J = 12.6$ Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 177.3, 108.4, 99.1, 95.2, 82.0, 81.2, 79.4, 75.1, 73.3, 72.7, 72.3, 71.5, 71.1, 70.0, 69.3, 67.3, 66.8, 60.4, 60.1, 50.6, 49.3, 49.2, 40.1, 27.7, 20.8. ESIHRMS calculated for $\text{C}_{23}\text{H}_{45}\text{N}_4\text{O}_{15}$ $[\text{M}+\text{H}]^+$, 617.2881; found, 617.2891.

4',6'-O-Benzylidene-penta-*N*-benzyloxycarbonyl-5''-O-(2,4,6-triisopropylbenzenesulfonyl)-paromomycin (106). A stirred solution of **105** (2 g, 1.46 mmol) in pyridine (15 mL) was treated with 2,4,6-triisopropylbenzenesulfonyl chloride (4.4 g, 14.56 mmol) (92 mg, 0.48 mmol) at RT. After stirring for 72 h at RT the reaction was quenched with methanol (15 mL) and concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with CHCl_3/IPA (19:1) to give desired product **106** (1.4 g, 58%). $[\alpha]_{\text{D}}^{\text{RT}} +27.7$ (c 1.2, MeOH). ESIHRMS calculated for $\text{C}_{85}\text{H}_{101}\text{N}_5\text{O}_{26}\text{NaS}$ $[\text{M}+\text{Na}]^+$, 1662.6353; found, 1662.6373.

4',6'-O-Benzylidenepenta-*N*-benzyloxycarbonyl-5''-deoxy-5''-azido paromomycin (107). To a stirred solution of **106** (1.2 g, 0.73 mmol) in DMF (10 mL) was added sodium azide (951 mg, 14.64 mmol). The reaction mixture was stirred for 2 h at 80 $^{\circ}\text{C}$ and concentrated under to dryness under reduced pressure. The residue was dissolved in acetone (50 mL) and the precipitate was filtered off. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with CHCl_3/IPA (19:1) to give the desired product **107** (800 mg, 78%). $[\alpha]_{\text{D}}^{\text{RT}} +34.5$ (c 1.0, MeOH). ESIHRMS calculated for $\text{C}_{70}\text{H}_{78}\text{N}_8\text{O}_{23}\text{Na}$ $[\text{M}+\text{Na}]^+$, 1421.5078; found, 1421.5051.

4',6'-O-Benzylidenepenta-N-benzyloxycarbonyl-5"-deoxy-5"-amino paromomycin (108). To a stirred solution of **107** (600 mg, 0.43 mmol) in a mixture of THF and water (1:1, 12 mL) was trimethylphosphine (1M in THF, 0.9 mL, 0.9 mmol). After stirring for 2 h at 80 °C the reaction was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH (18:1) to give desired product **108** (500 mg, 85%). $[\alpha]_{\text{D}}^{\text{RT}} +34.5$ (*c* 1.0, MeOH). ESIHRMS calculated for C₇₀H₈₁N₆O₂₃ [M+H]⁺, 1373.5353; found, 1373.5349.

4',6'-O-Benzylidenepenta-N-benzyloxycarbonyl-5"-deoxy-5"-formamido paromomycin (109). A stirred solution of **108** (250 mg, 0.18 mmol) in DCM (3 mL) was treated with formic acetic anhydride (3 mL) at RT. The reaction mixture was stirred for 2 h and then concentrated to dryness under reduced pressure. The residue was dissolved in MeOH (5 mL) and to this solution was added aq. NaHCO₃ (5 mL) at RT. The mixture was stirred for 1 h at RT and concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH (49:1) to give desired product **109** (175 mg, 69%). $[\alpha]_{\text{D}}^{\text{RT}} +44.3$ (*c* 0.53, MeOH). ESIHRMS calculated for C₇₁H₈₀N₆O₂₄Na [M+Na]⁺, 1423.5122; found, 1423.5095.

5"-Deoxy-5"-formamido paromomycin acetate salt (110). To a stirred suspension of Pd(OH)₂/C (160 mg, prewashed with glacial acetic acid) in water (2 mL) was added a solution of **18** (80 mg, 0.06 mmol) in dioxane (2 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (45 psi) for 8 h, filtered, concentrated under reduced pressure, and purified by Sephadex C-25 column chromatography (0.6% NH₄OH). The product containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to give the desired product **110** as the peracetate salt (25 mg, 26%). $[\alpha]_{\text{D}}^{\text{RT}} +44.0$ (*c* 0.8, H₂O). ¹H

NMR (600 MHz, D₂O) δ 7.89 (s, 1H), 5.50 (d, J = 2.7 Hz, 1H), 5.07 (d, J = 1.5 Hz, 1H), 5.00 (br s, 1H), 4.15 (t, J = 4.9 Hz, 1H), 4.08 – 4.05 (m, 1H), 4.05 – 4.01 (m, 1H), 4.00 – 3.92 (m, 2H), 3.78 (t, J = 9.0 Hz, 1H), 3.72 – 3.59 (m, 3H), 3.58 – 3.47 (m, 3H), 3.44 (t, J = 9.3 Hz, 1H), 3.40 (dd, J_1 = 14.5 Hz, J_2 = 3.5 Hz, 1H), 3.35 – 3.19 (m, 5H), 3.16 (dd, J_1 = 13.5 Hz, J_2 = 6.1 Hz, 1H), 3.13 – 3.04 (m, 2H), 2.21 (dt, J_1 = 12.5 Hz, J_2 = 4.2 Hz, 1H), 1.76 (s, 15H), 1.59 (q, J = 12.5 Hz, 1H). ¹³C NMR (150 MHz, D₂O) δ 177.4, 164.8, 109.5, 95.3, 95.0, 83.9, 79.7, 77.2, 76.7, 74.1, 72.9, 71.8, 70.0, 68.9, 68.7, 67.4, 67.2, 60.1, 53.3, 50.6, 49.4, 48.8, 40.3, 39.3, 27.8, 20.8. ESIHRMS calculated for C₂₄H₄₇N₆O₁₄ [M+H]⁺, 643.3150; found, 643.3145.

Penta-*N*-benzyloxycarbonyl-3',4',6'-hexa-*O*-acetyl-5"-deoxy-5"-acetamido paromomycin (111). A stirred solution of **108** (80 mg, 0.06 mmol) in DCM (1 mL) was treated with an excess of acetic anhydride (1 mL) at RT. The reaction mixture was stirred for 24 h at RT. LCMS analysis of the reaction mixture showed incomplete acetamide formation and at the same time 4',6'-*O*-benzylidene acetal was cleaved completely. Then to the reaction mixture was added pyridine (1 mL) and the reaction mixture was stirred for additional 18 hours at RT. Then the reaction mixture was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with CHCl₃/MeOH (49:1) to give the desired product **111** (75 mg, 79%). $[\alpha]_D^{RT}$ +33.8 (c 0.94, MeOH). ESIHRMS calculated for C₇₉H₉₂N₆O₃₁Na [M+Na]⁺, 1643.5705; found, 1643.5653.

5"-Acetamido-5"-deoxy- paromomycin-acetate salt (112). To a stirred solution of **111** (75 mg, 0.05 mmol) in MeOH (1 mL) was added NaOMe (20 mg, 0.37 mmol) at RT. After stirring for 2 h, the reaction mixture was neutralized with Amberlyst (H-form), filtered, and concentrated under reduced pressure. The residue as dissolved in dioxane (1.5 ml) and was added to a stirred suspension of Pd/C (75 mg) in 10% AcOH (0.75 mL). The reaction mixture was stirred under a

hydrogen atmosphere (45 psi) for 18 h, filtered, concentrated under reduced pressure, and purified by Sephadex C-25 column chromatography (0.8% NH_4OH). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to give the desired product **112** as the peracetate salt (26 mg, 49%). $[\alpha]_{\text{D}}^{\text{RT}} +55.7$ (c 0.87, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.43 (d, $J = 3.7$ Hz, 1H), 5.04 (d, $J = 3.2$ Hz, 1H), 4.97 (br s, 1H), 4.10 (t, $J = 5.4$ Hz, 1H), 4.03 – 3.97 (m, 2H), 3.95 – 3.88 (m, 2H), 3.74 (t, $J = 9.5$ Hz, 1H), 3.66 – 3.56 (m, 3H), 3.55 – 3.44 (m, 3H), 3.42 (t, $J = 9.8$ Hz, 1H), 3.32 (dd, $J_1 = 14.5$ Hz, $J_2 = 3.6$ Hz, 1H), 3.29 – 3.22 (m, 2H), 3.20 (t, $J = 9.1$ Hz, 1H), 3.16 (dd, $J_1 = 10.6$ Hz, $J_2 = 4.0$ Hz, 1H), 3.15 – 3.09 (m, 2H), 3.09 – 3.01 (m, 2H), 2.18 (dt, $J_1 = 12.7$ Hz, $J_2 = 4.1$ Hz, 1H), 1.72 (s, 18H), 1.56 (q, $J = 12.7$ Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 177.9, 174.4, 109.4, 95.4, 95.2, 83.8, 79.9, 77.5, 77.0, 74.1, 73.0, 71.8, 70.0, 68.9, 68.8, 67.4, 67.1, 60.1, 53.4, 50.6, 49.4, 48.8, 41.0, 40.2, 27.8, 21.9, 21.1. ESIHRMS calculated for $\text{C}_{25}\text{H}_{49}\text{N}_6\text{O}_{14}$ $[\text{M}+\text{H}]^+$, 657.3307; found, 657.3273.

4',6'-O-Benzylidene-penta-N-benzyloxycarbonyl-5''-deoxy-5''-(3-N-benzylureido) paromomycin (113). A stirred solution of **108** (100 mg, 0.07 mmol) in DCM (1 mL) was treated with benzyl isocyanate (50 μL) at RT. The reaction mixture was stirred for 2 h, quenched with MeOH (5 mL), and concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel eluting with $\text{CHCl}_3/\text{MeOH}$ (24:1) to give the desired product **113** (70 mg, 64%). $[\alpha]_{\text{D}}^{\text{RT}} +16.8$ (c 0.9, MeOH). ESIHRMS calculated for $\text{C}_{78}\text{H}_{87}\text{N}_7\text{O}_{24}\text{Na}$ $[\text{M}+\text{Na}]^+$, 1528.5700; found, 1527.5717.

5''-Deoxy-5''-ureido-paromomycin acetate salt (114). To a stirred suspension of Pd/C (90 mg) in 80% AcOH (0.5 mL) was added a solution of **113** (30 mg, 0.02 mmol) in 80% AcOH (1.0 mL) at RT. The reaction mixture was stirred under a hydrogen atmosphere (45 psi) for 12 h, filtered, concentrated under reduced pressure, and purified by Sephadex C-25 column

chromatography (0.7% NH_4OH). The product-containing fractions were concentrated under reduced pressure. The residue was dissolved in 10% AcOH and freeze dried to give the desired product **114** as the peracetate salt (8 mg, 42%). $[\alpha]_{\text{D}}^{25} +18.5$ (c 0.27, H_2O). ^1H NMR (600 MHz, D_2O) δ 5.54 (d, $J = 3.4$ Hz, 1H), 5.12 (d, $J = 3.3$ Hz, 1H), 5.05 (br s, 1H), 4.20 (t, $J = 5.2$ Hz, 1H), 4.12 – 4.09 (m, 1H), 4.09 – 4.06 (m, 1H), 4.03 – 3.95 (m, 2H), 3.83 (t, $J = 9.4$ Hz, 1H), 3.74 – 3.64 (m, 3H), 3.60 (br s, 1H), 3.61 – 3.50 (m, 2H), 3.49 (t, $J = 9.8$ Hz, 1H), 3.38 – 3.31 (m, 2H), 3.31 – 3.24 (m, 3H), 3.21 (dd, $J_1 = 13.8$ Hz, $J_2 = 6.2$ Hz, 1H), 3.18 – 3.10 (m, 3H), 2.26 (dt, $J_1 = 12.7$ Hz, $J_2 = 4.2$ Hz, 1H), 1.82 (s, 15H), 1.64 (q, $J = 12.7$ Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 177.1, 161.2, 109.6, 95.5, 95.4, 84.1, 80.4, 77.7, 77.0, 74.1, 73.0, 71.9, 70.0, 69.0, 68.8, 67.5, 67.3, 60.2, 53.5, 50.7, 49.4, 48.9, 41.4, 40.3, 27.9, 20.6. ESIHRMS calculated for $\text{C}_{24}\text{H}_{48}\text{N}_7\text{O}_{14}$ $[\text{M}+\text{H}]^+$, 658.3259; found, 658.3258.

2,3-Dimethyl-*N*-hydroxymaleimide (122). To a mixture of 2,3-dimethylmaleic anhydride **120** (3.0 g, 23.8 mmol), hydroxylamine hydrochloride (8.3 g, 118.9 mmol), and sodium acetate (9.7 g, 118.9 mmol) was added a 1:1 mixture of water and ethanol (100 mL). The reaction mixture was heated to reflux for 0.25 h. After cooling to RT the reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were washed with water followed by brine, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was then triturated with hexane to give the desired product **122** (2.5 g, 74%), mp 129-131 $^\circ\text{C}$, lit 126-127 $^\circ\text{C}$, as an off white solid. ^1H NMR (600 MHz, CDCl_3) δ 8.19 (br s, 1H), 1.94 (s, 6H); ^{13}C NMR (150 MHz, CDCl_3) δ 168.9, 135.6, 8.8.

2,3-Diphenyl-*N*-hydroxymaleimide (123). To a mixture of 2,3-diphenyl maleic anhydride **121** (1.0 g, 4.0 mmol), hydroxylamine hydrochloride (1.4 g, 20.0 mmol), and sodium acetate (1.6 g, 20.0 mmol) was added a 1:1 mixture of water and ethanol (100 mL). The reaction mixture was

heated to reflux for 0.25 h. After cooling to RT the reaction mixture was diluted with water (25 mL) and extracted with ethyl acetate (3 x 25 mL). The combined organic layers were washed with water followed by brine, dried over Na₂SO₄, and concentrated under reduced. The residue was purified by column chromatography on silica gel eluting with ethyl acetate/hexane (2:3) to give the desired product **123** (0.85 g, 80%), mp 203-204 °C, lit 196-197 °C, as a yellow solid. ¹H NMR (600 MHz, CD₃OD) δ 7.41 (d, *J* = 7.4 Hz, 4H), 7.39 – 7.34 (m, 2H), 7.32 (t, *J* = 7.5 Hz, 4H); ¹³C NMR (150 MHz, CD₃OD) δ 167.2, 133.8, 129.6, 128.4, 128.1. ESIHRMS calculated for C₁₆H₁₁NO₃Na [M+Na]⁺, 288.0637; found, 288.0650.

2-Methyl-3-phenyl maleic anhydride (126). To a mixture of sodium pyruvate **124** (5.0 g, 45.4 mmol) and phenylacetic acid **125** (6.2 g, 45.4 mmol) was added acetic anhydride (80 mL). The reaction mixture was refluxed for 3 h then cooled to RT and poured to ice cold water (150 mL). The water layer was extracted with ethyl acetate (3 x 75 mL). The combined organic layers were washed with water followed by brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with ethyl acetate/hexane (1:9) to give a yellow solid. This yellow solid was then recrystallized from ethyl acetate/hexane (1:8) to give 2-methyl 3-phenyl-maleic anhydride **126** (4.5 g, 53%), mp 99-101 °C, lit 98-100 °C as an off white solid. ¹H NMR (600 MHz, CDCl₃) δ 7.68 – 7.59 (m, 2H), 7.57 – 7.42 (m, 3H), 2.30 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 166.2, 164.8, 139.9, 138.7, 131.0, 129.4, 128.9, 127.4, 10.8.

2-Methyl-3-phenyl-*N*-hydroxymaleimide (127). To a mixture of 2-methyl-3-phenyl maleic anhydride **126** (3.4 g, 18.1 mmol), hydroxylamine hydrochloride (6.3 g, 90.4 mmol), and sodium acetate (7.4 g, 90.4 mmol) was added a 1:1 mixture of water and ethanol (100 mL). After stirring for 1 hour at RT the reaction mixture was diluted with water (50 mL) and extracted with

ethyl acetate (3 x 50 mL). The combined organic layers were washed with water followed by brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was then triturated with hexane to give the desired product **3** (3.3 g, 90 %), mp 164-166 °C, as a yellow solid. ¹H NMR (600 MHz, CDCl₃+ CD₃OD) δ 7.49 (d, *J* = 7.0 Hz, 2H), 7.43 – 7.33 (m, 3H), 2.12 (s, 3H); ¹³C NMR (150 MHz, CDCl₃+ CD₃OD) δ 168.5, 167.6, 134.9, 134.1, 129.7, 129.3, 128.5, 128.4, 9.8. ESIHRMS calculated for C₁₁H₈NO₃ [M-H]⁻, 202.0504; found, 202.0524.

***N*-acetoxycyclohexene-1,2-dicarboximide (129).** To a mixture of cyclohexene-1,2-dicarboxylic anhydride **128** (2.0 g, 13.1 mmol), hydroxylamine hydrochloride (4.6 g, 65.7 mmol), and sodium acetate (5.4 g, 65.7 mmol) was added a 1:1 mixture of water and ethanol (60 mL). The reaction mixture was refluxed for 1 h, then cooled to RT, diluted with water (30 mL), and extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with water followed by brine, dried over Na₂SO₄, and concentrated under reduced pressure to yield an off-white solid. This solid was dissolved in acetic anhydride (20 mL) and heated to reflux for 4 h. Then the reaction mixture was cooled to RT and poured to ice cold water (50 mL). The water layer was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with water followed by brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with ethyl acetate/hexane (1:9 to 2:8) to give *N*-acetoxycyclohexene-1,2-dicarboximide **129** (0.7 g, 25%) as a viscous gel. ¹H NMR (600 MHz, CDCl₃) δ 2.40-2.30 (m, 4H), 2.29 (s, 3H), 1.85-1.70 (m, 4H); ¹³C NMR (150 MHz, CDCl₃+ CD₃OD) δ 167.3, 165.3, 140.4, 20.9, 20.0.

***N*-Hydroxycyclohexene-1,2-dicarboximide (130).** To a stirred solution of *N*-acetoxycyclohexene-1,2-dicarboximide **129** (0.7 g, 3.3 mmol) in chloroform (15 mL) was added benzylamine (0.44 mL, 4.0 mmol) at RT. After stirring for 12 h at RT, the reaction mixture was

vigorously shaken with saturated aq. NaHCO_3 (40 mL). The separated aq. NaHCO_3 layer was acidified with 2N HCl and extracted with ethyl acetate (3 x 25 mL). The combined organic layers were washed with water followed by brine, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with ethyl acetate/hexane (2:8 to 3:7) to give the desired product **130** (0.35 g, 62%), mp 136-137 $^\circ\text{C}$, lit 139-141 $^\circ\text{C}$, as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 7.84 (br s, 1H), 2.34 – 2.27 (m, 4H), 1.79 – 1.71 (m, 4H); ^{13}C NMR (150 MHz, CDCl_3) δ 167.8, 139.8, 21.0, 19.9. ESIHRMS calculated for $\text{C}_8\text{H}_{10}\text{NO}_3$ $[\text{M}+\text{H}]^+$, 168.0661; found, 168.0653.

2,3-Dimethyl-N-trifloxymaleimide (131). A stirred solution of compound **122** (0.8 g, 5.7 mmol) in DCM (20 mL) was treated with pyridine (1.4 mL, 17.0 mmol) at RT and cooled to 0 $^\circ\text{C}$ before triflic anhydride (1.1 mL, 6.8 mmol) was added dropwise while stirring. After stirring for 1 additional h at 0 $^\circ\text{C}$ the reaction was quenched with water (50 mL). The DCM layer was separated and water layer was extracted with DCM (2 x 30 mL). The combined organic layers were washed with water followed by brine, dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was dissolved in hexane and filtered to remove any insoluble material. The hexane layer was concentrated under reduced pressure to obtain the desired product **5** (1.2 g, 77%) as a viscous gel. ^1H NMR (600 MHz, CDCl_3) δ 2.05 (s, 6H); ^{13}C NMR (150 MHz, CDCl_3) δ 165.0, 139.2, 118.4 (q, $J_{\text{CF}} = 322.6$ Hz, CF_3), 9.3. ESIHRMS calculated for $\text{C}_7\text{H}_6\text{NO}_5\text{F}_3\text{NaS}$ $[\text{M}+\text{Na}]^+$, 295.9816; found, 295.9811.

2,3-Diphenyl-N-trifloxymaleimide (115). A stirred solution of compound **123** (0.45 g, 1.7 mmol) in DCM (10 mL) was treated with pyridine (0.4 mL, 5.1 mmol) at RT and cooled to 0 $^\circ\text{C}$ before triflic anhydride (0.34 mL, 2.0 mmol) was added dropwise while stirring. After stirring for 1 additional hour at 0 $^\circ\text{C}$ the reaction was quenched with water (20 mL). The DCM layer was

separated and the water layer was extracted with DCM (2 x 20 mL). The combined organic layers were washed with water followed by brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was dissolved in hexane and filtered to remove any insoluble material. The filtrate was concentrated under reduced pressure to obtain the desired product **115** (0.6 g, 89%), mp 99-100 °C, as a yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 7.53 – 7.48 (m, 4H), 7.48 – 7.42 (m, 2H), 7.39 (t, *J* = 7.6 Hz, 4H); ¹³C NMR (150 MHz, CDCl₃) δ 164.0, 136.1, 131.2, 129.8, 128.9, 127, 118.6 (q, *J*_{CF} = 322.7 Hz, CF₃). ESIHRMS calculated for C₁₇H₁₀NO₅F₃NaS [M+Na]⁺, 420.0129; found, 420.0121.

2-Methyl-3-phenyl-N-trifloxymaleimide (132). A stirred solution of compound **127** (1.0 g, 4.9 mmol) in DCM (30 mL) was treated with pyridine (1.2 mL, 14.8 mmol) at RT and cooled to -25 °C before a solution of triflic anhydride (0.34 mL, 2.0 mmol) in DCM (10 mL) was added dropwise over a period of 30 minutes while stirring. After stirring for 1 additional hour at -25 °C the reaction mixture was quenched with water (40 mL). The DCM layer was separated and the water layer was extracted with DCM (2 x 30 mL). The combined organic layers were washed with water followed by brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was dissolved in hexane and filtered to discard any insoluble material. The hexane layer was concentrated under reduced pressure to obtain the desired product **132** (1.1 g, 67%) as viscous gel and used for subsequent reactions without any further purification. ¹H NMR (600 MHz, CDCl₃) δ 7.63 – 7.58 (m, 2H), 7.53 – 7.48 (m, 3H), 2.30 (s, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 165.0, 164.1, 138.0, 137.1, 131.1, 129.5, 129.0, 127.3, 118.5 (q, *J*_{CF} = 322.7 Hz, CF₃), 10.8. ESIHRMS calculated for C₁₂H₈NO₅F₃NaS [M+Na]⁺, 357.9973; found, 357.9971.

***N*-Trifloxycyclohexene-1,2-dicarboxylic acid imide (133).** A stirred solution of compound **130** (0.35 g, 2.1 mmol) in DCM (10 mL) was treated with pyridine (0.5 mL, 6.3 mmol) at RT and cooled to 0 °C before triflic anhydride (0.42 mL, 2.5 mmol) was added dropwise while stirring. After stirring for 1 additional hour at 0 °C the reaction was quenched with water (20 mL). The DCM layer was separated and the water layer was extracted with DCM (2 x 20 mL). The combined organic layers were washed with water followed by brine, dried over Na₂SO₄ and concentrated under reduced pressure to obtain the desired product **133** (0.36 g, 57%) as viscous gel. Due to the unstable nature of this compound it was used for subsequent reactions without any further purification. ¹H NMR (600 MHz, CDCl₃) δ 2.46 – 2.33 (m, 4H), 1.84 – 1.79 (m, 4H); ¹³C NMR (150 MHz, CDCl₃) δ 163.9, 142.9, 118.5 (q, *J*_{CF} = 322.7 Hz, CF₃), 20.6, 20.4.

General reaction protocol for the formation of 3-*N*-alkyl pyrimidin-2,4-diones. To a stirred solution of *N*-trifloxyimide (0.8 mmol) in DMF (2 mL) was added K₂CO₃ (1.6 mmol) at RT. To this mixture was added a solution of amine (0.5 mmol) in DMF (0.5 mL) dropwise at RT. After stirring for 4 h at RT the reaction was quenched with water (15 mL). The water layer was extracted with ethyl acetate (3 x 15 mL). The combined organic layers were washed with water followed by brine, dried over Na₂SO₄, and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel to give the desired 3-*N*-alkyl pyrimidin-2,4-dione.

3-*N*-(2-Hydroxy-2-phenylethyl)-5,6-dimethylpyrimidine-2,4-(1*H*,3*H*)-dione (138). This compound was prepared according to the general procedure using compound **131** (299 mg, 1.1 mmol), 2-amino-1-phenylethanol **134** (100 mg, 0.7 mmol), and K₂CO₃ (302 mg, 2.2 mmol). Purification by column chromatography eluting with ethyl acetate/hexane (1:5 to 9:1) afforded the desired product **138** (100 mg, 53%), mp 182-183 °C, as a white solid. ¹H NMR (600 MHz,

CDCl₃+CD₃OD) δ 7.37 (d, J = 7.5 Hz, 2H), 7.27 (t, J = 7.6 Hz, 2H), 7.19 (t, J = 7.3 Hz, 1H), 4.93 (dd, J = 9.4, 2.8 Hz, 1H), 4.17 (dd, J = 13.8, 9.4 Hz, 1H), 4.09 – 4.01 (m, 1H), 2.05 (s, 3H), 1.83 (s, 3H); ¹³C NMR (150 MHz, CDCl₃+CD₃OD) δ 165.2, 152.5, 145.8, 141.7, 128.3, 127.6, 152.7, 106.1, 72.7, 48.4, 16.4, 10.3. ESIHRMS calculated for C₁₄H₁₆N₂O₃Na [M+Na]⁺, 283.1059; found, 283.1070.

3-Cyclohexyl-5,6-dimethylpyrimidine-2,4-(1*H*,3*H*)-dione (139). This compound was prepared according to the general procedure using compound **131** (413 mg, 1.5 mmol), cyclohexylamine **135** (100 mg, 1.0 mmol), and K₂CO₃ (418 mg, 3.0 mmol). Purification by column chromatography eluting with ethyl acetate/hexane (1:5 to 2:3) gave the desired product **139** (125 mg, 56%), mp 215-216.5 °C, as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 10.47 (s, 1H), 4.75 (br t, J = 11.4 Hz, 1H), 2.37 (q, J = 12.0 Hz, 2H), 2.11 (s, 3H), 1.87 (s, 3H), 1.79 (d, J = 13.1 Hz, 2H), 1.61 (dd, J = 26.1, 12.0 Hz, 3H), 1.34 (q, J = 13.1 Hz, 2H), 1.24 – 1.11 (m, 1H); ¹³C NMR (150 MHz, CDCl₃) δ 164.4, 152.9, 144.5, 106.6, 53.4, 28.6, 26.3, 25.4, 16.5, 10.6. ESIHRMS calculated for C₁₂H₁₈N₂O₂Na [M+Na]⁺, 245.1266; found, 245.1283.

3-(1-Phenylethyl)-5,6-dimethylpyrimidine-2,4-(1*H*,3*H*)-dione (140). This compound was prepared according to the general procedure using compound **131** (338 mg, 1.2 mmol), (±)- α -methyl benzylamine **136** (100 mg, 0.8 mmol), and K₂CO₃ (342 mg, 2.5 mmol). Purification by column chromatography eluting with ethyl acetate/hexane (1:5 to 3:2) afforded the desired product **140** (80 mg, 40 %), mp 150-152 °C, as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 10.52 (s, 1H), 7.38 (d, J = 7.7 Hz, 2H), 7.26 (t, J = 7.7 Hz, 2H), 7.19 (t, J = 7.3 Hz, 1H), 6.27 (q, J = 7.0 Hz, 1H), 1.93 (s, 3H), 1.88 (s, 3H), 1.83 (d, J = 7.1 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 164.1, 152.5, 145.0, 140.5, 127.9, 127.2, 126.8, 106.4, 49.9, 16.4, 15.7, 10.6. ESIHRMS calculated for C₁₄H₁₆N₂O₂Na [M+Na]⁺, 267.1109; found, 267.1123.

3-(3,4-Dimethoxyphenethyl)-5,6-diphenylpyrimidine-2,4-(1*H*,3*H*)-dione (118). This compound was prepared according to the general procedure using compound **115** (329 mg, 0.8 mmol), 3,4-dimethoxyphenethylamine **116** (100 mg, 0.5 mmol) and K₂CO₃ (229 mg, 1.6 mmol). Purification by column chromatography eluting with ethyl acetate/hexane (1:3 to 7:3) afforded the desired product **118** (120 mg, 51%), mp 212-213.5 °C, as an off white solid. ¹H NMR (600 MHz, CDCl₃) δ 10.05 (br s, 1H), 7.35 – 7.30 (m, 1H), 7.29 – 7.18 (m, 7H), 7.11 (dd, *J* = 7.5, 1.6 Hz, 2H), 6.82 – 6.75 (m, 3H), 4.15 – 4.10 (vr m, 2H), 3.86 (s, 3H), 3.83 (s, 3H), 2.97 – 2.77 (vr m, 2H); ¹³C NMR (150 MHz, CDCl₃) δ 163.1, 152.0, 148.9, 147.7, 147.4, 132.5, 132.4, 131.3, 131.1, 130.1, 128.9, 128.5, 128.0, 127.5, 121.0, 112.7, 112.2, 111.3, 55.93, 55.88, 42.8, 33.3. ESIHRMS calculated for C₂₆H₂₄N₂O₄Na [M+Na]⁺, 451.1634; found, 451.1649.

***N*-(3,4-dimethoxyphenethyl)-3-[3-(3,4-dimethoxyphenethyl)ureido]-2,3-diphenylacrylamide (119).** This compound was isolated as a byproduct (25 mg, 7%) during the synthesis of compound **118**, mp 235-237 °C, as an off white solid. ¹H NMR (600 MHz, CDCl₃) δ 12.09 (br s, 1H), 7.12 – 6.92 (m, 8H), 6.89 – 6.81 (m, 2H), 6.78 (d, *J* = 7.4 Hz, 1H), 6.72 – 6.61 (m, 3H), 6.55 (s, 1H), 6.50 (d, *J* = 7.3 Hz, 1H), 5.39 (br s, 1H), 3.85 (br s, 1H), 3.85 (s, 6H), 3.83 (s, 3H), 3.80 (s, 3H), 3.48 – 3.30 (vr m, 4H), 2.73 – 2.58 (vr m, 4H). ¹³C NMR (150 MHz, CDCl₃) δ 169.7, 153.9, 151.1, 149.0, 148.9, 147.7, 146.6, 135.8, 135.7, 132.1, 131.4, 131.0, 128.5, 128.3, 127.6, 127.2, 127.1, 120.6, 120.5, 111.9, 111.7, 111.4, 110.6, 55.9, 55.85, 55.81, 40.8, 35.7, 35.0. ESIHRMS calculated for C₃₆H₃₉N₃O₆Na [M+Na]⁺, 632.2737; found, 632.2710.

3-Benzyl-5,6-diphenylpyrimidine-2,4-(1*H*,3*H*)-dione (141). This compound was prepared according to the general procedure using compound **115** (556 mg, 1.4 mmol), benzylamine **137** (100 mg, 0.9 mmol), and K₂CO₃ (387 mg, 2.8 mmol). Purification by column chromatography eluting with ethyl acetate/hexane (1:9 to 3:7) afforded the desired product **141**

(180 mg, 54 %), mp 222.5-224 °C, as an off-white solid. ¹H NMR (600 MHz, CDCl₃) δ 10.50 (br s, 1H), 7.45 – 7.40 (m, 2H), 7.35 (t, *J* = 7.3 Hz, 1H), 7.28 (t, *J* = 7.7 Hz, 2H), 7.26 – 7.22 (m, 5H), 7.22 – 7.19 (m, 3H), 7.09 (dd, *J* = 7.3, 2.0 Hz, 2H), 5.08 (s, 2H); ¹³C NMR (150 MHz, CDCl₃) δ 163.1, 152.4, 147.7, 136.7, 132.6, 132.4, 131.4, 130.1, 129.7, 129.0, 128.4, 128.3, 128.0, 127.7, 127.4, 112.7, 44.3. ESIHRMS calculated for C₂₃H₁₈N₂O₂Na [M+Na]⁺, 377.1266; found, 377.1282.

3-(1-Phenylethyl)-5,6-diphenylpyrimidine-2,4-(1*H*,3*H*)-dione (142). This compound was prepared according to the general procedure using compound **115** (491 mg, 1.2 mmol), (±)-α-methyl benzylamine **136** (100 mg, 0.8 mmol), and K₂CO₃ (342 mg, 2.5 mmol). Purification by column chromatography eluting with ethyl acetate/hexane (3:7 to 4:1) afforded the desired product **142** (50 mg, 16%), mp 234-235.5 °C, as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 10.27 (br s, 1H), 7.39 – 7.32 (m, 3H), 7.29 (t, *J* = 7.7 Hz, 2H), 7.24 – 7.16 (m, 8H), 7.11 – 7.06 (m, 2H), 6.28 (q, *J* = 6.9 Hz, 1H), 1.80 (d, *J* = 7.2 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 161.3, 151.9, 147.7, 140.0, 132.7, 132.4, 131.4, 130.0, 128.9, 128.4, 127.96, 127.90, 127.87, 127.3, 127.1, 112.6, 50.6, 15.9. ESIHRMS calculated for C₂₄H₂₀N₂O₂Na [M+Na]⁺, 391.1422; found, 391.1434.

3-(3,4-Dimethoxyphenethyl)-6-methyl-5-phenylpyrimidine-2,4-(1*H*,3*H*)-dione (143) and 3-(3,4-Dimethoxyphenethyl)-5-methyl-6-phenylpyrimidine-2,4-(1*H*,3*H*)-dione (144). These compounds were prepared according to the general procedure using compound **132** (277 mg, 0.8 mmol), 3,4-dimethoxyphenethylamine **116** (100 mg, 0.5 mmol), and K₂CO₃ (229 mg, 1.7 mmol). Purification by column chromatography eluting with ethyl acetate/hexane (1:5 to 7:3) afforded desired products **143** (60 mg), mp 222-224 °C, and **144** (50 mg), mp 119.5-193 °C, as off-white solids with a combined yield of 54%.

3-(3,4-Dimethoxyphenethyl)-6-methyl-5-phenylpyrimidine-2,4-(1*H*,3*H*)-dione (143).

¹H NMR (600 MHz, (CD₃)₂SO) δ 11.19 (br s, 1H), 7.34 (t, *J* = 7.4 Hz, 2H), 7.28 (t, *J* = 7.3 Hz, 1H), 7.17 (d, *J* = 7.3 Hz, 2H), 6.84 (d, *J* = 8.1 Hz, 1H), 6.73 (s, 1H), 6.69 (d, *J* = 8.1 Hz, 1H), 3.98 – 3.92 (vr m, 2H), 3.70 (s, 3H), 3.68 (s, 3H), 2.76 – 2.69 (vr m, 2H), 1.92 (s, 3H); ¹³C NMR (150 MHz, (CD₃)₂SO) δ 162.6, 151.0, 149.0, 147.9, 147.8, 134.0, 131.5, 131.3, 128.3, 127.6, 120.9, 112.8, 112.4, 111.3, 55.9, 55.7, 41.7, 33.1, 17.6. ESIHRMS calculated for C₂₁H₂₂N₂O₄Na [M+Na]⁺, 389.1477; found, 389.1481.

3-(3,4-Dimethoxyphenethyl)-5-methyl-6-phenylpyrimidine-2,4-(1*H*,3*H*)-dione (144).

¹H NMR (600 MHz, (CD₃)₂SO) δ 11.07 (br s, 1H), 7.47 (dd, *J* = 6.5, 3.6 Hz, 3H), 7.43 – 7.37 (m, 2H), 6.84 (t, *J* = 9.5 Hz, 1H), 6.77 (d, *J* = 1.8 Hz, 1H), 6.73 (dd, *J* = 8.1, 1.8 Hz, 1H), 4.03 – 3.93 (vr m, 2H), 3.71 (s, 3H), 3.69 (s, 3H), 2.78 – 2.71 (vr m, 2H), 1.70 (s, 3H); ¹³C NMR (150 MHz, (CD₃)₂SO) δ 164.2, 151.0, 149.0, 147.8, 147.7, 132.8, 131.5, 130.2, 129.0, 128.9, 120.9, 112.7, 112.4, 105.1, 55.9, 55.8, 41.8, 33.1, 12.2. ESIHRMS calculated for C₂₁H₂₂N₂O₄Na [M+Na]⁺, 389.1477; found, 389.1477.

3-Benzyl-6-methyl-5-phenylpyrimidine-2,4(1*H*,3*H*)-dione (145) and 3-Benzyl-5-methyl-6-phenylpyrimidine-2,4-(1*H*,3*H*)-dione (146). These compounds were prepared according to the general procedure using compound **132** (469 mg, 1.4 mmol), benzylamine **137** (100 mg, 0.9 mmol), and K₂CO₃ (387 mg, 2.8 mmol). Purification by column chromatography eluting with ethyl acetate/hexane (1:5 to 7:3) afforded the desired products **145** (85 mg), mp 288-290 °C, and **146** (55 mg), mp 210 -212 °C, as off-white solids with a combined yield of 51%.

3-Benzyl-6-methyl-5-phenylpyrimidine-2,4(1*H*,3*H*)-dione (145). ¹H NMR (600 MHz, (CD₃)₂SO) δ 11.30 (br s, 1H), 7.34 (t, *J* = 7.4 Hz, 3H), 7.30 – 7.25 (m, 5H), 7.23 – 7.17 (m, 4H), 4.96 (s, 2H), 1.94 (s, 3H); ¹³C NMR (150 MHz, (CD₃)₂SO) δ 162.8, 151.2, 148.4, 137.9, 133.9,

131.3, 128.7, 128.3, 128.1, 127.6, 127.5, 111.3, 43.4, 17.7. ESIHRMS calculated for $C_{18}H_{16}N_2O_2Na$ $[M+Na]^+$, 315.1109; found, 315.1124.

3-Benzyl-5-methyl-6-phenylpyrimidine-2,4-(1*H*,3*H*)-dione (146). 1H NMR (600 MHz, $(CD_3)_2SO$) δ 11.16 (br s, 1H), 7.49 – 7.42 (m, J = 13.5, 6.5, 3.2 Hz, 5H), 7.33 – 7.26 (m, 4H), 7.25 – 7.20 (m, 1H), 4.99 (s, 2H), 1.70 (s, 3H); ^{13}C NMR (150 MHz, $(CD_3)_2SO$) δ 164.3, 151.1, 148.0, 137.8, 132.8, 130.2, 129.0, 128.9, 128.7, 128.2, 127.5, 105.1, 43.5, 12.2. ESIHRMS calculated for $C_{18}H_{16}N_2O_2Na$ $[M+Na]^+$, 315.1109; found, 315.1107.

3-Cyclohexyl-6-methyl-5-phenylpyrimidine-2,4-(1*H*,3*H*)-dione (147) and 3-Cyclohexyl-5-methyl-6-phenylpyrimidine-2,4-(1*H*,3*H*)-dione (148). These compounds were prepared according to the general procedure using compound **132** (507 mg, 1.5 mmol), cyclohexylamine **135** (100 mg, 1.0 mmol) and K_2CO_3 (387 mg, 3.0 mmol). Purification by column chromatography eluting with ethyl acetate/hexane (1:9 to 2:3) afforded the desired products **147** (60 mg), mp 292-294 $^{\circ}C$, and **148** (40 mg), mp 233 -235 $^{\circ}C$, as off-white solids with a combined yield of 35%.

3-Cyclohexyl-6-methyl-5-phenylpyrimidine-2,4-(1*H*,3*H*)-dione (147). 1H NMR (600 MHz, $CDCl_3+CD_3OD$) δ ; 7.30 (t, J = 7.5 Hz, 2H), 7.27 – 7.21 (m, 5.0 Hz, 1H), 7.13 (d, J = 7.5 Hz, 2H), 4.68 (br t, J = 11.8 Hz, 1H), 2.25 (q, J =11.4 Hz, 2H), 1.92 (s, 3H), 1.73 (d, J = 13.1 Hz, 2H), 1.62 – 1.50 (m, 3H), 1.25 (q, J = 13.0 Hz, 2H), 1.17 – 1.05 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3+CD_3OD$) δ 163.8, 151.9, 146.5, 133.0, 130.6, 128.2, 127.5, 113.1, 53.8, 28.5, 26.2, 25.2, 17.0. ESIHRMS calculated for $C_{17}H_{20}N_2O_2Na$ $[M+Na]^+$, 307.1422; found, 307.1411.

3-Cyclohexyl-5-methyl-6-phenylpyrimidine-2,4-(1*H*,3*H*)-dione (148). 1H NMR (600 MHz, $CDCl_3+CD_3OD$) δ 7.44 – 7.36 (m, 3H), 7.36 – 7.29 (m, 2H), 7.25 (s, 1H), 4.68 (br t, J = 10.7 Hz, 1H), 2.25 (d, J = 11.4 Hz, 2H), 1.79 (s, 3H), 1.73 (d, J = 12.5 Hz, 2H), 1.62 – 1.47 (m,

3H), 1.27 (q, $J=13.2$ Hz, 2H), 1.15 – 1.04 (m, 1H); ^{13}C NMR (150 MHz, $\text{CDCl}_3+\text{CD}_3\text{OD}$) δ 165.3, 151.9, 146.5, 132.5, 130.0, 128.6, 128.1, 106.9, 53.7, 28.4, 26.2, 25.2, 11.8. ESIHRMS calculated for $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_2\text{Na}$ $[\text{M}+\text{Na}]^+$, 307.1422; found, 307.1414.

3-(3,4-Dimethoxyphenethyl)-5,6,7,8-tetrahydroquinazoline-2,4-(1*H*,3*H*)-dione (149).

This compound was prepared according to the general procedure using compound **133** (150 mg, 0.5 mmol), 3,4-dimethoxyphenethylamine **116** (60 mg, 0.3 mmol), and K_2CO_3 (138 mg, 1.0 mmol). Purification by column chromatography eluting with ethyl acetate/hexane (3:7 to 4:1) afforded the desired product **149** (70 mg, 63 %), mp 229.5-231 $^\circ\text{C}$, as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 10.17 (s, 1H), 6.88 – 6.71 (m, 3H), 4.18 – 4.01 (vr m, 2H), 3.85 (s, 3H), 3.83 (s, 3H), 2.93 – 2.78 (vr m, 2H), 2.42 – 2.34 (m, 4H), 1.80-1.67 (m, 4H); ^{13}C NMR (150 MHz, CDCl_3) δ 163.5, 152.6, 148.9, 147.6, 146.4, 131.3, 120.8, 112.1, 111.2, 108.0, 55.88, 55.79, 42.0, 33.4, 26.2, 21.7, 21.3, 21.1. ESIHRMS calculated for $\text{C}_{18}\text{H}_{22}\text{N}_2\text{O}_4\text{Na}$ $[\text{M}+\text{Na}]^+$, 353.1477; found, 353.1468.

3-Cyclohexyl-5,6,7,8-tetrahydroquinazoline-2,4-(1*H*,3*H*)-dione (150). This compound was prepared according to the general procedure using compound **133** (165 mg, 0.5 mmol), cyclohexylamine **135** (37 mg, 0.4 mmol) and K_2CO_3 (152 mg, 1.1 mmol). Purification by column chromatography eluting with ethyl acetate/hexane (3:7 to 4:1) afforded the desired product **150** (50 mg, 55 %), mp 296-298 $^\circ\text{C}$ as a white solid. ^1H NMR (600 MHz, $\text{CDCl}_3+\text{CD}_3\text{OD}$) δ 4.61 (br t, $J = 12.2$ Hz, 1H), 2.32 – 2.13 (m, 6H), 1.70 (d, $J = 13.5$ Hz, 2H) 1.66 – 1.43 (m, 7H), 1.22 (q, $J = 13.0$ Hz, 2H) 1.13–1.01 (m, 1H); ^{13}C NMR (150 MHz, $\text{CDCl}_3+\text{CD}_3\text{OD}$) δ 164.4, 152.3, 146.2, 108.0, 53.3, 28.6, 26.3, 25.9, 25.3, 21.7, 21.3, 21.1. ESIHRMS calculated for $\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_2\text{Na}$ $[\text{M}+\text{Na}]^+$, 271.1422; found, 271.1425.

Bacterial strains. Clinical isolates of *E. coli*, *S. aureus* and *P. aeruginosa* were obtained from the Diagnostic Department, Institute of Medical Microbiology, University of Zurich. MIC values were determined by broth microdilution assays as described.³⁹

Recombinant microorganisms. The construction of these strains derived from single rRNA allelic *M. smegmatis* Δ rrnB, has been described previously.^{68,77}

Cell-free translation assays. Rabbit reticulocyte lysate (Promega), S-30 extracts and purified ribosomes were used for cell-free translation assays as described previously.³⁹ Firefly luciferase mRNA was used as reporter to monitor translation activity. Luminescence was measured using a luminometer Flx800 (Bio-Tek Instruments).

REFERENCES

- (1) Porter, J. R. *Bacteriol. Rev.* **1976**, *40*, 260-269.
- (2) Austrian, R. *Bacteriol. Rev.* **1960**, *24*, 261.
- (3) Walsh, C. *Antibiotics: actions, origins, resistance*; American Society for Microbiology (ASM), 2003.
- (4) Global T.B. Report 2016. World Health Organization, Geneva, **2016**.
- (5) Williams, K. *J. R. Soc. Med.* **2009**, *102*, 343-348.
- (6) Bosch, F.; Rosich, L. *Pharmacology* **2008**, *82*, 171-179.
- (7) Limbird, L. E. *Mol. Interv.* **2004**, *4*, 326.
- (8) Waksman, S. A. *Mycologia* **1947**, 565-569.
- (9) Lloyd, N. C.; Morgan, H. W.; Nicholson, B. K.; Ronimus, R. S. *Angew. Chem., Int. Ed. Engl.* **2005**, *44*, 941-944.
- (10) Houbraken, J.; Frisvad, J. C.; Samson, R. A. *IMA Fungus: The Global Mycological Journal* **2011**, *2*, 87.
- (11) Waksman, S. A. *Antimicrob. Agents Chemother.* **1965**, *5*, 9.
- (12) Jackson, J.; Chen, C.; Buising, K. *Curr. Opin. Infect. Dis.* **2013**, *26*, 516-525.
- (13) Vakulenko, S. B.; Mobashery, S. *Clin. Microbiol. Rev.* **2003**, *16*, 430-450.
- (14) Fosso, M. Y.; Li, Y.; Garneau-Tsodikova, S. *MedChemComm* **2014**, *5*, 1075-1091.
- (15) World Health Organization, Geneva, **2007**.
- (16) Goodman, L. S. *Goodman and Gilman's the pharmacological basis of therapeutics*; McGraw-Hill New York, 1996; Vol. 1549.
- (17) Hanberger, H.; Edlund, C.; Furebring, M.; G. Giske, C.; Melhus, Å.; Nilsson, L. E.; Petersson, J.; Sjölin, J.; Ternhag, A.; Werner, M. *Scand. J. Infect. Dis.* **2013**, *45*, 161-175.

- (18) Carter, A. P.; Clemons, W. M.; Brodersen, D. E.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V. *Nature* **2000**, *407*, 340-348.
- (19) de Loubresse, N. G.; Prokhorova, I.; Holtkamp, W.; Rodnina, M. V.; Yusupova, G.; Yusupov, M. *Nature* **2014**, *513*, 517-522.
- (20) François, B.; Russell, R. J.; Murray, J. B.; Aboul-ela, F.; Masquida, B.; Vicens, Q.; Westhof, E. *Nucleic Acids Res.* **2005**, *33*, 5677-5690.
- (21) Peske, F.; Savelsbergh, A.; Katunin, V. I.; Rodnina, M. V.; Wintermeyer, W. *J. Mol. Biol.* **2004**, *343*, 1183-1194.
- (22) Feldman, M. B.; Terry, D. S.; Altman, R. B.; Blanchard, S. C. *Nat. Chem. Biol.* **2010**, *6*, 54-62.
- (23) Moazed, D.; Noller, H. F. *Nature* **1987**, *327*, 389-394.
- (24) Tsai, A.; Uemura, S.; Johansson, M.; Puglisi, E. V.; Marshall, R. A.; Aitken, C. E.; Korlach, J.; Ehrenberg, M.; Puglisi, J. D. *Cell Rep.* **2013**, *3*, 497-508.
- (25) Arya, D. P. *Aminoglycoside antibiotics: from chemical biology to drug discovery*; John Wiley & Sons, 2007; Vol. 5.
- (26) Wong, C.-H. *Carbohydrate-based drug discovery*; John Wiley & Sons, 2003; Vol. 1.
- (27) Magnet, S.; Blanchard, J. S. *Chem. Rev.* **2005**, *105*, 477-498.
- (28) Houghton, J. L.; Green, K. D.; Chen, W.; Garneau-Tsodikova, S. *ChemBioChem* **2010**, *11*, 880-902.
- (29) Ramirez, M. S.; Tolmasky, M. E. *Drug Resist. Updates* **2010**, *13*, 151-171.
- (30) Labby, K. J.; Garneau-Tsodikova, S. *Future Med. Chem.* **2013**, *5*, 1285-1309.
- (31) Yang, L.; Ye, X.-S. *Curr. Top. Med. Chem.* **2010**, *10*, 1898-1926.
- (32) ECDC, E. *Stockholm: European Center for Disease Prevention and Control* **2009**.

- (33) Umezawa, S. *Adv. Carbohydr. Chem. Biochem.* **1974**, *30*, 111-182.
- (34) Haddad, J.; Kotra, L.; Mobashery, S.; Marcel Dekker, Inc: 2001.
- (35) Chandrika, N. T.; Garneau-Tsodikova, S. *MedChemComm* **2016**, *7*, 50-68.
- (36) Aggen, J. B.; Armstrong, E. S.; Goldblum, A. A.; Dozzo, P.; Linsell, M. S.; Gliedt, M. J.; Hildebrandt, D. J.; Feeney, L. A.; Kubo, A.; Matias, R. D. *Antimicrob. Agents Chemother.* **2010**, *54*, 4636-4642.
- (37) Zhanel, G. G.; Lawson, C. D.; Zelenitsky, S.; Findlay, B.; Schweizer, F.; Adam, H.; Walkty, A.; Rubinstein, E.; Gin, A. S.; Hoban, D. J. *Expert Rev. Anti-Infect. Ther.* **2012**, *10*, 459-473.
- (38) Ryden, R.; Moore, B. J. *Antimicrob. Chemother.* **1977**, *3*, 609-613.
- (39) Matt, T.; Ng, C. L.; Lang, K.; Sha, S.-H.; Akbergenov, R.; Shcherbakov, D.; Meyer, M.; Duscha, S.; Xie, J.; Dubbaka, S. R. *Proc. Natl. Acad. Sci. USA.* **2012**, *109*, 10984-10989.
- (40) Meyer, M.; Freihofer, P.; Scherman, M.; Teague, J.; Lenaerts, A.; Böttger, E. C. *Antimicrob. Agents Chemother.* **2014**, *58*, 6938-6941.
- (41) Pittengbr, R.; Wolfe, E.; Hoehn, M.; Marks, P. N.; Daily, W.; McGuire, J. *Antibiot. Chemother.* **1953**, *3*, 1268-1278.
- (42) Jana, S.; Deb, J. *Appl. Biochem. Biotechnol.* **2006**, *70*, 140-150.
- (43) Stromgaard, K.; Krogsgaard-Larsen, P.; Madsen, U. *Textbook of drug design and discovery*; CRC Press, 2009.
- (44) Nelson, D. L.; Lehninger, A. L.; Cox, M. M. *Lehninger principles of biochemistry*; Macmillan, 2008.
- (45) Beringer, M.; Bruell, C.; Xiong, L.; Pfister, P.; Bieling, P.; Katunin, V. I.; Mankin, A. S.; Böttger, E. C.; Rodnina, M. V. *J. Biol. Chem.* **2005**, *280*, 36065-36072.

- (46) Hobbie, S. N.; Pfister, P.; Brüll, C.; Westhof, E.; Böttger, E. C. *Antimicrob. Agents Chemother.* **2005**, *49*, 5112-5118.
- (47) Hobbie, S. N.; Pfister, P.; Bruell, C.; Sander, P.; François, B.; Westhof, E.; Böttger, E. C. *Antimicrob. Agents Chemother.* **2006**, *50*, 1489-1496.
- (48) Hobbie, S.; Bruell, C.; Kalapala, S.; Akshay, S.; Schmidt, S.; Pfister, P.; Böttger, E. *Biochimie* **2006**, *88*, 1033-1043.
- (49) Tor, Y. *ChemBioChem* **2003**, *4*, 998-1007.
- (50) Thomas, J. R.; Hergenrother, P. J. *Chem. Rev.* **2008**, *108*, 1171-1224.
- (51) Ogle, J. M.; Ramakrishnan, V. *Annu. Rev. Biochem.* **2005**, *74*, 129-177.
- (52) Shandrick, S.; Zhao, Q.; Han, Q.; Ayida, B. K.; Takahashi, M.; Winters, G. C.; Simonsen, K. B.; Vourloumis, D.; Hermann, T. *Angew. Chem.* **2004**, *116*, 3239-3244.
- (53) Kaul, M.; Barbieri, C. M.; Pilch, D. S. *J. Am. Chem. Soc.* **2004**, *126*, 3447-3453.
- (54) Forge, A.; Schacht, J. *Audiology and Neurotology* **2000**, *5*, 3-22.
- (55) Böttger, E. C.; Schacht, J. *Hear. Res.* **2013**, *303*, 12-19.
- (56) Huth, M.; Ricci, A.; Cheng, A. *Int. J. Otolaryngol.* **2011**, *2011*.
- (57) Rybak, M. J.; Abate, B. J.; Kang, S. L.; Ruffing, M. J.; Lerner, S. A.; Drusano, G. L. *Antimicrob. Agents Chemother.* **1999**, *43*, 1549-1555.
- (58) Mingeot-Leclercq, M.-P.; Tulkens, P. M. *Antimicrob. Agents Chemother.* **1999**, *43*, 1003-1012.
- (59) Kishore, B.; Kallay, Z.; Lambricht, P.; Laurent, G.; Tulkens, P. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 867-874.
- (60) Janknegt, R. *Pharm. World. Sci.* **1990**, *12*, 81-90.
- (61) O'Neil, W. G. *Toxicology* **2008**, *249*, 91-96.

- (62) Tange, R. *Ziekenhuisfarmacie* **1987**, 3, 15-17.
- (63) Lautermann, J.; McLaren, J.; Schacht, J. *Hear. Res.* **1995**, 86, 15-24.
- (64) Huy, P. T. B.; Bernard, P.; Schacht, J. *J. Clin. Invest.* **1986**, 77, 1492.
- (65) Beidler, L. M. *Handbook of sensory physiology*; Springer, 1971; Vol. 4.
- (66) Fausti, S. A.; Rappaport, B. Z.; Schechter, M. A.; Frey, R. H.; Ward, T. T.; Brummett, R. *E. Am. J. Otolaryngol.* **1984**, 5, 177-182.
- (67) Pfister, P.; Hobbie, S.; Brüll, C.; Corti, N.; Vasella, A.; Westhof, E.; Böttger, E. *J. Mol. Biol.* **2005**, 346, 467-475.
- (68) Hobbie, S. N.; Bruell, C. M.; Akshay, S.; Kalapala, S. K.; Shcherbakov, D.; Böttger, E. *C. Proc. Natl. Acad. Sci. USA.* **2008**, 105, 3244-3249.
- (69) Matt, T.; Akbergenov, R.; Shcherbakov, D.; Böttger, E. *C. Isr. J. Chem.* **2010**, 50, 60-70.
- (70) Prezant, T. R.; Agapian, J. V.; Bohlman, M. C.; Bu, X.; Öztas, S.; Qiu, W.-Q.; Arnos, K. S.; Cortopassi, G. A.; Jaber, L.; Rotter, J. I. *Nat. Genet.* **1993**, 4, 289-294.
- (71) Hershko, C. *Mol. Aspects Med.* **1992**, 13, 113-165.
- (72) Tanswell, A. K.; Freeman, B. A. *New Horiz.* **1995**, 3, 330-341.
- (73) Sha, S.-H.; Schacht, J. *Lab. Invest.* **1999**, 79, 807-814.
- (74) Rodnina, M. V.; Wintermeyer, W.; Green, R. *Ribosomes Structure, Function, and Dynamics*; Springer Science & Business Media, 2011.
- (75) Hobbie, S. N.; Akshay, S.; Kalapala, S. K.; Bruell, C. M.; Shcherbakov, D.; Böttger, E. *C. Proc. Natl. Acad. Sci. USA.* **2008**, 105, 20888-20893.
- (76) Pape, T.; Wintermeyer, W.; Rodnina, M. V. *Nat. Struct. Mol. Biol.* **2000**, 7, 104-107.
- (77) Hobbie, S. N.; Kalapala, S. K.; Akshay, S.; Bruell, C.; Schmidt, S.; Dabow, S.; Vasella, A.; Sander, P.; Böttger, E. *C. Nucleic Acids Res.* **2007**, 35, 6086-6093.

- (78) Nikaido, H.; Pagès, J.-M. *FEMS Microbiol. Rev.* **2012**, *36*, 340-363.
- (79) Li, X.-Z.; Plésiat, P.; Nikaido, H. *Clin. Microbiol. Rev.* **2015**, *28*, 337-418.
- (80) Macfarlane, E. L.; Kwasnicka, A.; Hancock, R. E. *Microbiology* **2000**, *146*, 2543-2554.
- (81) Fernández, L.; Gooderham, W. J.; Bains, M.; McPhee, J. B.; Wiegand, I.; Hancock, R. E. *Antimicrob. Agents Chemother.* **2010**, *54*, 3372-3382.
- (82) Kwon, D. H.; Lu, C.-D. *Antimicrob. Agents Chemother.* **2006**, *50*, 1615-1622.
- (83) Nowicki, E. M.; O'Brien, J. P.; Brodbelt, J. S.; Trent, M. S. *Mol. Microbiol.* **2015**, *97*, 166-178.
- (84) Bansal-Mutalik, R.; Nikaido, H. *Proc. Natl. Acad. Sci. USA.* **2014**, *111*, 4958-4963.
- (85) Miller, M. H.; Edberg, S. C.; Mandel, L. J.; Behar, C. F.; Steigbigel, N. H. *Antimicrob. Agents Chemother.* **1980**, *18*, 722-729.
- (86) Fernández, L.; Hancock, R. E. *Clin. Microbiol. Rev.* **2012**, *25*, 661-681.
- (87) Sarathy, J. P.; Dartois, V.; Lee, E. J. D. *Pharmaceuticals* **2012**, *5*, 1210-1235.
- (88) Danilchanka, O.; Pavlenok, M.; Niederweis, M. *Antimicrob. Agents Chemother.* **2008**, *52*, 3127-3134.
- (89) Faller, M.; Niederweis, M.; Schulz, G. E. *Science* **2004**, *303*, 1189-1192.
- (90) Poole, K. J. *Antimicrob. Chemother.* **2005**, *56*, 20-51.
- (91) Li, X.-Z.; Poole, K.; Nikaido, H. *Antimicrob. Agents Chemother.* **2003**, *47*, 27-33.
- (92) Poelarends, G. J.; Mazurkiewicz, P.; Konings, W. N. *Biochim. Biophys. Acta* **2002**, *1555*, 1-7.
- (93) Begum, A.; Rahman, M. M.; Ogawa, W.; Mizushima, T.; Kuroda, T.; Tsuchiya, T. *Microbiol. Immunol.* **2005**, *49*, 949-957.
- (94) De Stasio, E.; Moazed, D.; Noller, H.; Dahlberg, A. *EMBO J.* **1989**, *8*, 1213.

- (95) Prammananan, T.; Sander, P.; Brown, B. A.; Frischkorn, K.; Onyi, G. O.; Zhang, Y.; Böttger, E. C.; Wallace Jr, R. J. *J. Infect. Dis.* **1998**, *177*, 1573-1581.
- (96) Recht, M. I.; Douthwaite, S.; Puglisi, J. D. *EMBO J.* **1999**, *18*, 3133-3138.
- (97) Basso, L. A.; Blanchard, J. S. In *Resolving the Antibiotic Paradox*; Springer: 1998, p 115-144.
- (98) Springer, B.; Kidan, Y. G.; Prammananan, T.; Ellrott, K.; Böttger, E. C.; Sander, P. *Antimicrob. Agents Chemother.* **2001**, *45*, 2877-2884.
- (99) Unemo, M.; Golparian, D.; Skogen, V.; Olsen, A. O.; Moi, H.; Syversen, G.; Hjelmevoll, S. O. *Antimicrob. Agents Chemother.* **2013**, *57*, 1057-1061.
- (100) Wachino, J.-i.; Arakawa, Y. *Drug Resist. Updates* **2012**, *15*, 133-148.
- (101) Doi, Y.; Yokoyama, K.; Yamane, K.; Wachino, J.-i.; Shibata, N.; Yagi, T.; Shibayama, K.; Kato, H.; Arakawa, Y. *Antimicrob. Agents Chemother.* **2004**, *48*, 491-496.
- (102) Chow, J. W. *Clin. Infect. Dis.* **2000**, *31*, 586-589.
- (103) Beauclerk, A. A.; Cundliffe, E. *J. Mol. Biol.* **1987**, *193*, 661-671.
- (104) Yokoyama, K.; Doi, Y.; Yamane, K.; Kurokawa, H.; Shibata, N.; Shibayama, K.; Yagi, T.; Kato, H.; Arakawa, Y. *The Lancet* **2003**, *362*, 1888-1893.
- (105) Tada, T.; Miyoshi-Akiyama, T.; Kato, Y.; Ohmagari, N.; Takeshita, N.; Hung, N. V.; Phuong, D. M.; Thu, T. A.; Binh, N. G.; Anh, N. Q. *BMC Infect. Dis.* **2013**, *13*, 251.
- (106) Shoma, S.; Kamruzzaman, M.; Ginn, A. N.; Iredell, J. R.; Partridge, S. R. *Diagn. Microbiol. Infect. Dis.* **2014**, *78*, 93-97.
- (107) Galimand, M.; Courvalin, P.; Lambert, T. *Antimicrob. Agents Chemother.* **2003**, *47*, 2565-2571.

- (108) González-Zorn, B.; Teshager, T.; Casas, M.; Porrero, M. C.; Moreno, M. A.; Courvalin, P.; Domínguez, L. *Emerg. Infect. Dis.* **2005**, *11*, 954.
- (109) Livermore, D.; Mushtaq, S.; Warner, M.; Zhang, J.-C.; Maharjan, S.; Doumith, M.; Woodford, N. *J. Antimicrob. Chemother.* **2010**, *66*, 48-53.
- (110) Garneau-Tsodikova, S.; Labby, K. J. *MedChemComm* **2016**, *7*, 11-27.
- (111) Bacot-Davis, V. R.; Bassenden, A. V.; Berghuis, A. M. *MedChemComm* **2016**, *7*, 103-113.
- (112) Bassenden, A. V.; Rodionov, D.; Shi, K.; Berghuis, A. M. *ACS Chem. Biol.* **2016**, *11*, 1339-1346.
- (113) Wolf, E.; Vassilev, A.; Makino, Y.; Sali, A.; Nakatani, Y.; Burley, S. K. *Cell* **1998**, *94*, 439-449.
- (114) Wybenga-Groot, L. E.; Draker, K.-a.; Wright, G. D.; Berghuis, A. M. *Structure* **1999**, *7*, 497-507.
- (115) Vetting, M. W.; Magnet, S.; Nieves, E.; Roderick, S. L.; Blanchard, J. S. *Chemistry & Biology* **2004**, *11*, 565-573.
- (116) Vetting, M. W.; Hegde, S. S.; Javid-Majd, F.; Blanchard, J. S.; Roderick, S. L. *Nat. Struct. Mol. Biol.* **2002**, *9*, 653-658.
- (117) Vetting, M. W.; de Carvalho, L. P. S.; Yu, M.; Hegde, S. S.; Magnet, S.; Roderick, S. L.; Blanchard, J. S. *Arch. Biochem. Biophys.* **2005**, *433*, 212-226.
- (118) Kotra, L. P.; Haddad, J.; Mobashery, S. *Antimicrob. Agents Chemother.* **2000**, *44*, 3249-3256.
- (119) Resistance in *Pseudomonas*, I.-M. R. *Antimicrob. Agents Chemother.* **1999**, *43*, 727-737.
- (120) Gray, G. S.; Fitch, W. M. *Mol. Biol. Evol.* **1983**, *1*, 57-66.

- (121) Fong, D. H.; Berghuis, A. M. *EMBO J.* **2002**, *21*, 2323-2331.
- (122) McKay, G.; Thompson, P. R.; Wright, G. *Biochemistry* **1994**, *33*, 6936-6944.
- (123) Trieu-Cuot, P.; Courvalin, P. *Gene* **1983**, *23*, 331-341.
- (124) Cox, J. R.; McKay, G. A.; Wright, G. D.; Serpersu, E. H. *J. Am. Chem. Soc.* **1996**, *118*, 1295-1301.
- (125) Thompson, P. R.; Hughes, D. W.; Wright, G. D. *Biochemistry* **1996**, *35*, 8686-8695.
- (126) Young, P. G.; Walanj, R.; Lakshmi, V.; Byrnes, L. J.; Metcalf, P.; Baker, E. N.; Vakulenko, S. B.; Smith, C. A. *J. Bacteriol.* **2009**, *191*, 4133-4143.
- (127) Buynak, J. D. *Biochem. Pharmacol.* **2006**, *71*, 930-940.
- (128) Allen, N.; Alborn, W.; Hobbs, J.; Kirst, H. *Antimicrob. Agents Chemother.* **1982**, *22*, 824-831.
- (129) Gao, F.; Yan, X.; Baettig, O. M.; Berghuis, A. M.; Auclair, K. *Angew. Chem., Int. Ed. Engl.* **2005**, *44*, 6859-6862.
- (130) Boehr, D. D.; Draker, K.-a.; Koteva, K.; Bains, M.; Hancock, R. E.; Wright, G. D. *Chemistry & Biology* **2003**, *10*, 189-196.
- (131) Sucheck, S. J.; Wong, A. L.; Koeller, K. M.; Boehr, D. D.; Draker, K.-a.; Sears, P.; Wright, G. D.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 5230-5231.
- (132) Chang, C.-W. T.; Hui, Y.; Elchert, B.; Wang, J.; Li, J.; Rai, R. *Org. Lett.* **2002**, *4*, 4603-4606.
- (133) Rai, R.; Chen, H.-N.; Czyryca, P. G.; Li, J.; Chang, C.-W. T. *Org. Lett.* **2006**, *8*, 887-889.
- (134) Russell, R. J.; Murray, J. B.; Lentzen, G.; Haddad, J.; Mobashery, S. *J. Am. Chem. Soc.* **2003**, *125*, 3410-3411.

- (135) Bastida, A.; Hidalgo, A.; Chiara, J. L.; Torrado, M.; Corzana, F.; Pérez-Canadillas, J. M.; Groves, P.; Garcia-Junceda, E.; Gonzalez, C.; Jimenez-Barbero, J. *J. Am. Chem. Soc.* **2006**, *128*, 100-116.
- (136) Hanessian, S.; Maianti, J. P.; Matias, R. D.; Feeney, L. A.; Armstrong, E. S. *Org. Lett.* **2011**, *13*, 6476-6479.
- (137) Sati, G. C.; Shcherbakov, D.; Hobbie, S. N.; Vasella, A.; Böttger, E. C.; Crich, D. *ACS Infect. Dis.* **2017**, *3*, 368-377.
- (138) Qian, Y.; Guan, M.-X. *Antimicrob. Agents Chemother.* **2009**, *53*, 4612-4618.
- (139) Armstrong, E. S.; Kostrub, C. F.; Cass, R. T.; Moser, H. E.; Serio, A. W.; Miller, G. H. In *Antibiotic discovery and development*; Springer: 2012, p 229-269.
- (140) Maianti, J. P.; Hanessian, S. *MedChemComm* **2016**, *7*, 170-176.
- (141) Hainrichson, M.; Pokrovskaya, V.; Shallom-Shezifi, D.; Fridman, M.; Belakhov, V.; Shachar, D.; Yaron, S.; Baasov, T. *Bioorg. Med. Chem.* **2005**, *13*, 5797-5807.
- (142) Zhang, J.; Chiang, F.-I.; Wu, L.; Czyryca, P. G.; Li, D.; Chang, C.-W. T. *J. Med. Chem.* **2008**, *51*, 7563-7573.
- (143) Zhang, J.; Keller, K.; Takemoto, J. Y.; Bensaci, M.; Litke, A.; Czyryca, P. G.; Chang, C.-W. T. *J. Antibiot.* **2009**, *62*, 539-544.
- (144) Hanessian, S.; Saavedra, O. M.; Vilchis-Reyes, M. A.; Maianti, J. P.; Kanazawa, H.; Dozzo, P.; Matias, R. D.; Serio, A.; Kondo, J. *Chem. Sci.* **2014**, *5*, 4621-4632.
- (145) Maianti, J. P.; Kanazawa, H.; Dozzo, P.; Matias, R. D.; Feeney, L. A.; Armstrong, E. S.; Hildebrandt, D. J.; Kane, T. R.; Gliedt, M. J.; Goldblum, A. A. *ACS Chem. Biol.* **2014**, *9*, 2067-2073.

- (146) Watkins, D.; Kumar, S.; Green, K. D.; Arya, D. P.; Garneau-Tsodikova, S. *Antimicrob. Agents Chemother.* **2015**, *59*, 3899-3905.
- (147) Jin, Y.; Watkins, D.; Degtyareva, N. N.; Green, K. D.; Spano, M. N.; Garneau-Tsodikova, S.; Arya, D. P. *MedChemComm* **2016**, *7*, 164-169.
- (148) Hanessian, S.; Giguere, A.; Grzyb, J.; Maianti, J. P.; Saavedra, O. M.; Aggen, J. B.; Linsell, M. S.; Goldblum, A. A.; Hildebrandt, D. J.; Kane, T. R. *ACS Med. Chem. Lett.* **2011**, *2*, 924-928.
- (149) Duscha, S.; Boukari, H.; Shcherbakov, D.; Salian, S.; Silva, S.; Kendall, A.; Kato, T.; Akbergenov, R.; Perez-Fernandez, D.; Bernet, B. *mBio* **2014**, *5*, e01827-01814.
- (150) Nagabhushan, T. L.; Google Patents: 1976.
- (151) Davies, D. H.; Mallams, A. K.; Counelis, M.; Loebenberg, D.; Moss Jr, E. L.; Waitz, J. *A. J. Med. Chem.* **1978**, *21*, 189-193.
- (152) Hendrix, M.; Alper, P. B.; Priestley, E. S.; Wong, C. H. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 95-98.
- (153) Greenberg, W. A.; Priestley, E. S.; Sears, P. S.; Alper, P. B.; Rosenbohm, C.; Hendrix, M.; Hung, S.-C.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 6527-6541.
- (154) Morgenthaler, M.; Schweizer, E.; Hoffmann-Röder, A.; Benini, F.; Martin, R. E.; Jaeschke, G.; Wagner, B.; Fischer, H.; Bendels, S.; Zimmerli, D. *ChemMedChem* **2007**, *2*, 1100-1115.
- (155) Pathak, R.; Perez-Fernandez, D.; Nandurdikar, R.; Kalapala, S. K.; Böttger, E. C.; Vasella, A. *Helv. Chim. Acta* **2008**, *91*, 1533-1552.
- (156) Toda, S.; Nakagawa, S.; Naito, T.; Kawaguchi, H. *J. Antibiot.* **1983**, *36*, 87-91.
- (157) Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2007**, *9*, 3797-3800.

- (158) Potter, G. T.; Jayson, G. C.; Miller, G. J.; Gardiner, J. M. *J. Org. Chem.* **2016**, *81*, 3443-3446.
- (159) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897-2904.
- (160) Davies, J.; Benveniste, R. *Biochemistry* **1971**, *10*, 1787-1796.
- (161) Chun-bao, Z.; Sunada, A.; Ishikawa, J.; Ikeda, Y.; Kondo, S.; Hotta, K. *J. Antibiot.* **1999**, *52*, 889-894.
- (162) Cassinelli, G.; Julita, P.; Arcamone, F. *J. Antibiot.* **1978**, *31*, 382-384.
- (163) Williams, J. M. *Adv. Carbohydr. Chem. Biochem.* **1975**, *31*, 9-79.
- (164) Pathak, R.; Böttger, E. C.; Vasella, A. *Helv. Chim. Acta* **2005**, *88*, 2967-2985.
- (165) Autissier, D.; Barthelemy, P.; Mazieres, N.; Peyre, M.; Penasse, L. *J. Antibiot.* **1981**, *34*, 536-543.
- (166) Vicens, Q.; Westhof, E. *Biopolymers* **2003**, *70*, 42-57.
- (167) Perez-Fernandez, D.; Shcherbakov, D.; Matt, T.; Leong, N. C.; Kudyba, I.; Duscha, S.; Boukari, H.; Patak, R.; Dubbaka, S. R.; Lang, K. *Nat. Commun.* **2014**, *5*, 3112.
- (168) Kato, T.; Yang, G.; Teo, Y.; Juskeviciene, R.; Perez-Fernandez, D. b.; Shinde, H. M.; Salian, S.; Bernet, B.; Vasella, A.; Böttger, E. C. *ACS Infect. Dis.* **2015**, *1*, 479-486.
- (169) Alper, P. B.; Hendrix, M.; Sears, P.; Wong, C.-H. *J. Am. Chem. Soc.* **1998**, *120*, 1965-1978.
- (170) Hermann, T.; Westhof, E. *J. Med. Chem.* **1999**, *42*, 1250-1261.
- (171) Kandasamy, J.; Atia-Glikin, D.; Shulman, E.; Shapira, K.; Shavit, M.; Belakhov, V.; Baasov, T. *J. Med. Chem.* **2012**, *55*, 10630-10643.
- (172) Shalev, M.; Baasov, T. *MedChemComm* **2014**, *5*, 1092-1105.

- (173) Shalev, M.; Rozenberg, H.; Smolkin, B.; Nasereddin, A.; Kopelyanskiy, D.; Belakhov, V.; Schrepfer, T.; Schacht, J.; Jaffe, C. L.; Adir, N. *Nucleic Acids Res.* **2015**, *43*, 8601-8613.
- (174) Matsushita, T.; Chen, W.; Juskeviciene, R.; Teo, Y.; Shcherbakov, D.; Vasella, A.; Böttger, E. C.; Crich, D. *J. Am. Chem. Soc.* **2015**, *137*, 7706-7717.
- (175) Yan, R.-B.; Yuan, M.; Wu, Y.; You, X.; Ye, X.-S. *Bioorg. Med. Chem.* **2011**, *19*, 30-40.
- (176) Green, K. D.; Chen, W.; Houghton, J. L.; Fridman, M.; Garneau-Tsodikova, S. *ChemBioChem* **2010**, *11*, 119-126.
- (177) Fourmy, D.; Recht, M. I.; Puglisi, J. D. *J. Mol. Biol.* **1998**, *277*, 347-362.
- (178) Rather, P.; Orosz, E.; Shaw, K.; Hare, R.; Miller, G. *J. Bacteriol.* **1993**, *175*, 6492-6498.
- (179) Macinga, D. R.; Rather, P. N. *Front. Biosci.* **1999**, *4*, D132-D140.
- (180) Udou, T.; Mizuguchi, Y.; Wallace Jr, R. J. *Am. Rev. Respir. Dis.* **1987**, *136*, 338-343.
- (181) Ainsa, J. A.; Martin, C.; Gicquel, B.; Gomez-Lus, R. *Antimicrob. Agents Chemother.* **1996**, *40*, 2350-2355.
- (182) Hegde, S. S.; Javid-Majd, F.; Blanchard, J. S. *J. Biol. Chem.* **2001**, *276*, 45876-45881.
- (183) Wright, J. J.; Daniels, P. J. L.; Mallams, A. K.; Nagabhushan, T. L.; Scherico Ltd., Switz. . 1977, p 41 pp.
- (184) Wu, H.; Miller, G.; Blanco, M. G.; Hare, R.; Shaw, K. *Antimicrob. Agents Chemother.* **1997**, *41*, 2439-2447.
- (185) Kocabiyik, S.; Akalin, E.; Alaeddinoglu, N. G. *Int. J. Exp. Clin. Chemother.* **1989**, *2*, 118-124.
- (186) Shaw, K. J.; Sabatelli, F. J.; Naples, L.; Mann, P.; Hare, R. S.; Miller, G. H. *Methods Mol. Med.* **1998**, *15*, 555-577.

- (187) Legakis, N. J.; Velonaki, A.; Velonakis, E.; Lymberopoulou, T. *Chemioterapia* **1986**, *5*, 185-190.
- (188) Nagabhushan, T. L.; Miller, G.; Weinstein, M. *The aminoglycosides. Marcel Dekker, Inc., New York, NY* **1982**, 3-27.
- (189) Cassinelli, G.; Franceschi, G.; Di Colo, G.; Arcamone, F. *J. Antibiot.* **1978**, *31*, 379-381.
- (190) Smith, C. A.; Baker, E. N. *Curr. Drug. Targets Infect. Disord.* **2002**, *2*, 143-160.
- (191) Krimen, L. I. *Org. Synth.* **1988**, *50*, 8-9.
- (192) Ballestri, M.; Chatgililoglu, C.; Clark, K.; Griller, D.; Giese, B.; Kopping, B. *J. Org. Chem.* **1991**, *56*, 678-683.
- (193) Saegusa, T.; Kobayashi, S.; Ito, Y.; Yasuda, N. *J. Am. Chem. Soc.* **1968**, *90*, 4182-4182.
- (194) Barton, D. H.; Bringman, G.; Lamotte, G.; Motherwell, R. S. H.; Motherwell, W. B. *Tetrahedron Lett.* **1979**, *20*, 2291-2294.
- (195) Horton, D.; Philips, K. D. *Carbohydr. Res.* **1973**, *30*, 367-374.
- (196) Llewellyn, J.; Williams, J. *Carbohydr. Res.* **1973**, *28*, 339-350.
- (197) Lu, S. R.; Lai, Y. H.; Chen, J. H.; Liu, C. Y.; Mong, K. K. T. *Angew. Chem., Int. Ed. Engl.* **2011**, *50*, 7315-7320.
- (198) Davidson, R. N.; den Boer, M.; Ritmeijer, K. *Trans. R. Soc. Trop. Med. Hyg.* **2009**, *103*, 653-660.
- (199) Sittig, M. *Pharmaceutical manufacturing encyclopedia*; William Andrew Pub., 2015.
- (200) Kondo, J.; Koganei, M.; Maianti, J. P.; Ly, V. L.; Hanessian, S. *ChemMedChem* **2013**, *8*, 733-739.
- (201) Chen, W.; Matsushita, T.; Shcherbakov, D.; Boukari, H.; Vasella, A.; Böttger, E. C.; Crich, D. *MedChemComm* **2014**, *5*, 1179-1187.

- (202) Pfäffli, P. J.; Hixson, S. H.; Anderson, L. *Carbohydr. Res.* **1972**, *23*, 195-206.
- (203) Ferrier, R.; Hay, R.; Vethaviasar, N. *Carbohydr. Res.* **1973**, *27*, 55-61.
- (204) Swayze, E. E.; Hanessian, S.; Szychowski, J.; Adhikari, S. S.; Pachamuthu, K.; Wang, X.; Migawa, M. T.; Griffey, R. H.; Google Patents: 2013.
- (205) Torii, T.; Tsuchiya, T.; Umezawa, S.; Umezawa, H. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 1522-1526.
- (206) Kudyba, I.; Fernandez, D. P.; Böttger, E. C.; Vasella, A. *Carbohydr. Res.* **2007**, *342*, 499-519.
- (207) Blount, K. F.; Zhao, F.; Hermann, T.; Tor, Y. *J. Am. Chem. Soc.* **2005**, *127*, 9818-9829.
- (208) Hanessian, S.; Masse, R.; Capmeau, M.-L. *J. Antibiot.* **1977**, *30*, 893-896.
- (209) Culbertson, T.; Watson, D.; Haskell, T. *J. Antibiot.* **1973**, *26*, 790-793.
- (210) Degtyareva, N. N.; Gong, C.; Story, S.; Levinson, N. S.; Oyelere, A. K.; Green, K. D.; Garneau-Tsodikova, S.; Arya, D. P. *ACS Infect. Dis.* **2017**, *3*, 206-215.
- (211) Hanessian, S.; Takamoto, T.; Massé, R.; Patil, G. *Can. J. Chem.* **1978**, *56*, 1482-1491.
- (212) LaPlanche, L. A.; Rogers, M. T. *J. Am. Chem. Soc.* **1964**, *86*, 337-341.
- (213) Pawar, D. M.; Khalil, A. A.; Hooks, D. R.; Collins, K.; Elliott, T.; Stafford, J.; Smith, L.; Noe, E. A. *J. Am. Chem. Soc.* **1998**, *120*, 2108-2112.
- (214) Mizushima, S.-i.; Simanouti, T.; Nagakura, S.; Kuratani, K.; Tsuboi, M.; Baba, H.; Fujioka, O. *J. Am. Chem. Soc.* **1950**, *72*, 3490-3494.
- (215) Kotera, A.; Shibata, S.; Sone, K. *J. Am. Chem. Soc.* **1955**, *77*, 6183-6186.
- (216) Worsham Jr, J. E.; Hobbs, M. E. *J. Am. Chem. Soc.* **1954**, *76*, 206-208.
- (217) Leader, G. R.; Gormley, J. F. *J. Am. Chem. Soc.* **1951**, *73*, 5731-5733.
- (218) Suzuki, I. *Bull. Chem. Soc. Jpn.* **1962**, *35*, 540-551.

- (219) Miyazawa, T.; Shimanouchi, T.; Mizushima, S. i. *J. Chem. Phys.* **1956**, *24*, 408-418.
- (220) Miyazawa, T. *J. Mol. Spectrosc.* **1960**, *4*, 155-167.
- (221) Russell, R.; Thompson, H. *Spectrochim. Acta Mol. Biomol. Spectrosc.* **1956**, *8*, 138-141.
- (222) DeGraaf, D. E.; Sutherland, G. *J. Chem. Phys.* **1957**, *26*, 716-717.
- (223) Bellamy, L. J. In *The Infra-red Spectra of Complex Molecules*; Springer: 1975, p 231-262.
- (224) Huisgen, R.; Walz, H. *Eur. J. Inorg. Chem.* **1956**, *89*, 2616-2629.
- (225) Sati, G. C.; Crich, D. *Org. Lett.* **2015**, *17*, 4122-4124.
- (226) Vitaku, E.; Smith, D. T.; Njardarson, J. T. *J. Med. Chem.* **2014**, *57*, 10257-10274.
- (227) Taylor, R. D.; MacCoss, M.; Lawson, A. D. *J. Med. Chem.* **2014**, *57*, 5845-5859.
- (228) Ritchie, T. J.; Macdonald, S. J.; Peace, S.; Pickett, S. D.; Luscombe, C. N. *MedChemComm* **2012**, *3*, 1062-1069.
- (229) Lagoja, I. M. *Chem. Biodiv.* **2005**, *2*, 1-50.
- (230) Sankawa, U.; Shibata, S. *Chem. Pharm. Bull.* **1969**, *17*, 2020-2024.
- (231) Fields, E. K.; Behrend, S.; Meyerson, S.; Winzenburg, M.; Ortega, B.; Hall Jr, H. *J. Org. Chem.* **1990**, *55*, 5165-5170.
- (232) Barton, D.; Sammes, P.; Weingarten, G. *J. Chem. Soc. C* **1971**, 729-736.
- (233) Zinner, G.; Düerkop, E. *Arch. Pharm.* **1968**, *301*, 776-779.
- (234) Kayser, M. M.; Wipff, G. *Can. J. Chem.* **1982**, *60*, 1192-1198.
- (235) Wijnberg, J.; Schoemaker, H.; Speckamp, W. *Tetrahedron* **1978**, *34*, 179-187.
- (236) Franklin, E. C. *Chem. Rev.* **1934**, *14*, 219-250.
- (237) Bauer, L.; Exner, O. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 376-384.
- (238) Hurd, C. D.; Buess, C. M.; Bauer, L. *J. Org. Chem.* **1954**, *19*, 1140-1149.

- (239) Hurd, C. D.; Bauer, L. *J. Am. Chem. Soc.* **1954**, 76, 2791-2792.
- (240) Baldwin, J.; Barton, D.; Sutherland, J. *J. Chem. Soc.* **1965**, 1787-1798.
- (241) González-López, M.; Shaw, J. T. *Chem. Rev.* **2009**, 109, 164-189.
- (242) Crich, D.; Bowers, A. A. *Org. Lett.* **2007**, 9, 5323-5325.
- (243) Crich, D.; Sasaki, K.; Rahaman, M. Y.; Bowers, A. A. *J. Org. Chem.* **2009**, 74, 3886-3893.
- (244) Crich, D.; Rahaman, M. Y. *Tetrahedron* **2010**, 66, 6383-6390.
- (245) Crich, D.; Rahaman, M. Y. *J. Org. Chem.* **2009**, 74, 6792-6796.

ABSTRACT**ADDRESSING THE THREAT OF MULTIDRUG RESISTANT INFECTIOUS DISEASES BY SYNTHESIS OF NOVEL AMINOGLYCOSIDE ANTIBIOTICS**

by

GIRISH CHANDRA SATI**May 2018****Advisor:** Dr. David Crich**Major:** Chemistry**Degree:** Doctor of Philosophy

The ever-growing increase in multidrug resistant infectious diseases is one of the major cause of human mortality, and there is an inherent need for the development of new antibiotics. Since the discovery of streptomycin, AGAs have been playing a very important role in human therapy as highly potent broad-spectrum antibiotics and are listed as one of the critically important antimicrobials by WHO. AGAs act by inhibiting the bacterial protein synthesis and by targeting the A-site present in the small subunit of bacterial ribosome. The clinical use of AGAs is somewhat restricted due to their toxic effects (ototoxicity and nephrotoxicity) and emergence of resistant bacterial strains. However, with the increase in resistance to current antibiotics and significant hurdles in discovering a new class of antibiotics, researchers started to revisit the AGAs, leading to much interest in development of novel AGAs. The goal of this thesis is to utilize the well understood mechanism of action and mechanisms of resistance for the development of novel AGAs. This research work is mainly focused on the modification of neomycin B and paromomycin.

Chapter one introduces the problem of infectious diseases caused by bacteria, history of antibiotics and need for the development of new antibiotics. Then it discusses history of AGAs, their classification, mechanism of action, and toxic effects. It also discusses the common mechanisms of resistance adopted by bacteria and the recent strategies used to evade those resistance mechanisms.

Chapter two discusses a series of modifications made to neomycin B and the influence of each modification on antibacterial activity and ribosomal selectivity. Fourteen different neomycin B derivatives were synthesized and their antiribosomal and antibacterial activities were determined. These derivatives include modifications at 2'-, 4'-, 6'-, and 6''' positions. Newly synthesized compounds were also screened against ESKAPE pathogens and engineered strains of *E. Coli* carrying specific resistance determinants in order to determine their susceptibility to modifications by common AMEs.

Chapter three describes the modifications at 2'- and 5''- positions in paromomycin and their influence on antiribosomal activity and selectivity. The 2'-position is susceptible to modification by AAC-(2') and was mainly modified by alkylating the 2'-amino substituent. APH-(3', 5'') is one of the most important AME and has been known to modify most of the AGAs. There has been a lot of effort in the past to circumvent the action of this AME and it still remains a great challenge. This chapter also discusses the successful 5''-formamido and 5''-ureido modifications to paromomycin. These 5''-modifications provide an effective alternative for 5''-hydroxy substituent and are not susceptible to modification at this position by APH-(3', 5'').

In Chapter four, an efficient and facile method for the synthesis of highly substituted pyrimidine-2,4-dione derivatives was described. Pyrimidine-2,4-diones are important nitrogen-based heterocycles and present in biologically active natural products and pharmaceuticals. This

chapter discuss their synthesis from easily accessible and inexpensive maleic anhydrides and primary amines.

AUTOBIOGRAPHICAL STATEMENT

GIRISH CHANDRA SATI

EDUCATIONAL & PROFESSIONAL BACKGROUND:

2012 – Present	PhD in Chemistry, Department of Chemistry, Wayne State University
2016 – 2017	Co-Op Intern, Muscle Metabolism DPU, GlaxoSmithKline
2007 – 2012	Research Chemist, Drug Discovery Research, Panacea Biotec Limited
2005 – 2007	Master of Science, H.N.B. Garhwal University
2002 – 2005	Bachelor of Science, H.N.B. Garhwal University

RESEARCH PUBLICATIONS:

1. Facile Synthesis of 3-*N*-Alkyl Pyrimidin-2,4-diones from *N*-Sulfonyloxy Maleimides and Amines. **Girish C. Sati** and David Crich, *Org. Lett.*, **2015**, 17, 4122-4124.
2. *N6'*, *N6'''*, and *O4'* Modifications to Neomycin Affect Ribosomal Selectivity without Compromising Antibacterial Activity. **Girish C. Sati**, Dimitri Shcherbakov, Sven N. Hobbie, Andrea Vasella, Erik C. Böttger, and David Crich, *ACS Infect. Dis.*, **2017**, 3 (5), 368–377.
3. Dissecting the influence of two structural substituents on the differential neurotoxic effects of methamphetamine and mephedrone on dopamine nerve endings with the use of 4-methylmethamphetamine and methcathinone. John H. Anneken, Mariana Angoa-Perez, **Girish C. Sati**, David Crich and Donald M. Kuhn, *Journal of Pharmacology and Experimental Therapeutics*, **2017**, 360 (3), 417-423.
4. Neomycin and Paromomycin Derivatives. Provisional application for the patent EP17165623. D. Crich, E. C. Böttger, A. Vasella, S. Hobbie, A. R. Mandhapat, **Girish C. Sati**, A. Sonousi, G. Yang, M. Pirrone, T. Kato, and V. Sarpe.
5. Assessing the Role of Dopamine in the Differential Neurotoxicity Patterns of Methamphetamine, Mephedrone, Methcathinone and 4-Methylmethamphetamine. John H. Anneken, Mariana Angoa-Perez, **Girish C. Sati**, David Crich and Donald M. Kuhn, *Neuropharmacology*, **2017**, DOI: 10.1016/j.neuropharm.2017.08.033.